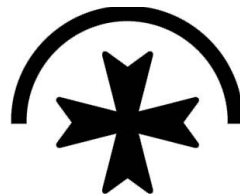


Predictors of cardiovascular disease in rheumatoid arthritis

**Thesis by
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2011**



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Science is but an image of the truth.

Francis Bacon 1561-1626

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Abbreviations

| | |
|-----------|---|
| ACR | American College of Rheumatology |
| Anti-CCP | Antibodies to Cyclic Citrullinated Peptide |
| BNP | B-type natriuretic peptide |
| CDAI | Clinical Disease Activity Index |
| cfPWV | Carotid-Femoral Pulse Wave Velocity |
| CHD | Coronary Heart Disease |
| CI | Confidence Interval |
| CRP | C - reactive Protein |
| CVD | Cardiovascular disease |
| CT | Computer Tomography |
| DAS | Disease Activity Score (also DAS28, based on 28 joint counts) |
| DMARD | Disease Modifying Anti-Rheumatic Drugs |
| ECG | Electrocardiogram |
| ESR | Erythrocyte Sedimentation Rate |
| Et al | and others |
| ESC | The European Society of Cardiology |
| EULAR | European League against Rheumatism |
| FDA | The United States Food and Drug Administration |
| FMD | Flow Mediated Dilatation |
| GTN | Glycerol trinitrate |
| HAQ | Health Assessment Questionnaire |
| HDL | High-Density Lipoprotein |
| HLA | Human Leukocyte Antigen |
| HR | Hazard Ratio |
| ICC | Intra class Correlation Coefficient |
| IHD | Ischemic Heart Disease |
| IMT | Intima Media Thickness |
| LDL | Low-density lipoprotein |
| MI | Myocardial Infarction |
| MTX | Methotrexate |
| NT-proBNP | N-terminal pro-B-type Natriuretic Peptide |

| | |
|-------|---|
| NHP | Nottingham Health Profile |
| NO | Nitric oxide |
| OR | Odds Ratio |
| ORAR | Oslo Rheumatoid Arthritis Register |
| PP | Pulse Pressure |
| PWV | Pulse Wave Velocity |
| RA | Rheumatoid Arthritis |
| RF | Rheumatoid Factor |
| SDAI | Simplified Diseases Activity Index |
| SD | Standard Deviation |
| SE | Shared Epitope |
| SJC | Swollen Joint Count |
| SPSS | Statistical Package for the Social Sciences |
| TJC | Tender Joint Count |
| TNF | Tumor Necrosis Factor |
| VAS | Visual Analogue Scale |
| vdHSS | van der Heijde modified Sharp Score |

List of papers

- I. Provan S, Angel K, Ødegård S, Mowinckel P, Atar D, Kvien TK. The association between NT-proBNP and disease activity in 238 patients with rheumatoid arthritis: A 10-year longitudinal study.
Arth Res Ther 2008; 10 (3): R70
- II. Provan S, Semb AG, Hisdal J, Stranden E, Agewall S, Dagfinrud H, Angel K, Atar D, Kvien TK. Remission is the mission in cardiovascular risk management in patients with rheumatoid arthritis: A cross-sectional comparative study.
In press Annals of the Rheumatic Diseases
- III. Provan S, Angel K, Semb AG, Mowinckel P, Agewall S, Atar D, Kvien TK
Early prediction of increased arterial stiffness in patients with chronic inflammation: A 15-year follow-up study of 108 rheumatoid arthritis patients.
In press Journal of Rheumatology
- IV. Provan S, Angel K, Semb AG, Atar D, Kvien TK. NT-proBNP predicts mortality in patients with rheumatoid arthritis.
Ann Rheum Dis. 2010 Feb; 69(2):325-31

1. Introduction

An individual receiving the diagnosis of rheumatoid arthritis (RA) will immediately have several years deducted from her or his statistical life expectancy. The mortality rate in patients with RA is 1.5-1.6 compared to that of the general public, and cardiovascular disease (CVD) accounts for 40-50% of the deaths [2,3]. Many of us will die from a CVD, but in people with RA, death often occurs at an earlier age.

Patients with RA may well have an unfavourable risk profile already at their first visit to the rheumatologist's office. The patient with RA has been shown to be at a considerably increased risk of developing a myocardial infarction (MI) already in the two years prior to fulfilling the American Congress of Rheumatology (ACR) diagnostic criteria for RA [4]. The patients with RA may have an unfavourable genetic profile, and there is evidence that they are more likely to have a close relative who has suffered from a myocardial infarction before the age of 60 [5]. Indeed the human leukocyte antigen (HLA) shared epitopes (SE) have been shown to be significant predictors of ischaemic heart disease (IHD) while also being associated with greater RA susceptibility and severity [6]. Common risk factors for CVD and RA such as smoking, or lack of physical exercise which is a consequence of RA and a risk factor for CVD, may also be of importance [5].

If we are to prevent cardiovascular events in patients with RA we must know which patients to focus our efforts on, and specifically which RA disease characteristics signal an increased risk of CVD. Biomarkers play an important role as predictors enabling us to audit the individual before the clinical disease becomes apparent. This thesis endeavours to make a small contribution in a rapidly expanding field and stands at the cross-road between cardiovascular medicine and rheumatology.

The transition from internal medicine to the field of rheumatology spurred my personal motivation for this work. As several authors drew my attention to the immunological similarities between the atherosclerotic lesion and synovitis, I hoped to find a PhD project that would allow me to develop my interest in both fields. The impetus to submerge myself in a PhD project came from listening to colleagues talk so animatedly about their fields of interest. The saying "The devil is in the details" sprung to my mind while considering project organizations and thesis work. However, I also had a little hope that there might be some truth behind the alternative idiom "God is in the details" (Ludwig Mies van der Rohe 1886-1969).

2. Background

2.1 Rheumatoid arthritis

2.1.1 Definition

RA is classified as a chronic, systemic, autoimmune disease. The major finding is a symmetrical swelling of the joints, predominantly affecting the wrists, metocarpophalangeal and proximal interphalangeal joints in the hands, and the metatarsophalangeal joints of the feet [7]. The classical symptoms are inflammatory joint pain and morning stiffness lasting more than one hour. The disease has the potential to become systemic causing constitutional symptoms such as shortness of breath due to pulmonary fibrosis or pericarditis, dry eyes and mouth due to secondary Sjögren's syndrome [8], and subcutaneous nodules. The 1987 ACR criteria has until very recently been used in order to classify patients. For the diagnosis of RA a patient must fulfil 4 out of 7 criterions; morning stiffness ≥ 1 hour, arthritis of ≥ 3 joint areas, arthritis of hand/wrist joints, symmetrical arthritis, rheumatic nodules, serum rheumatoid factor (RF) and radiographic changes [9].

2.1.2 Epidemiology

The incidence rate of RA in Oslo has been estimated across six consecutive inception cohorts, with an average of 25.7 cases in 100 000 patient years. Of the patients identified, 74.4% were female, and 25.6 % male [10]. The highest incidence rates were found in the oldest age groups (60-79) for both sexes (average of 60 cases pr 100 000 patient years). The overall prevalence of RA in Oslo in adults aged 20-79 has been estimated to be 0.44%, although women over 60 years have a prevalence that is at least three-fold this value [11]. Studies from the Norfolk Health Authority show similar findings with a female incidence rate of 36/100 000 and male incidence rate of 14/100 000. The peak onset was also in this study found to be in women aged 65-74, although the female: male ratio was attenuated with increasing age [12]. The prevalence of RA in the Norfolk cohort was however almost twice that in the Norwegian cohorts and studies from North America and Greece have also found a higher prevalence [12,13]. Meta-analyses of several epidemiological studies have indeed reported on divergent incidence and prevalence rates across geographical locations

and sampling dates, but despite differences there seems to be increasing support for the concept of a declining incidence of RA with a shift towards delayed disease onset [12-15].

2.1.3 Pathogenesis

The pathology of RA starts prior to clinical disease in many patients. Anti- cyclic citrullinated peptide antibodies (anti-CCP) can be detected many years preceding the onset of the disease, and assign a near 70% risk of developing the disease in patients who have a strong family history of RA [16-18]. Genetic risk factors such as the SE alleles interact with smoking, and probably other environmental factors, to increase the risk of developing anti-CCP antibodies [19]. Auto-antibodies towards IgG, termed the RF and until recently considered the most important serological biomarker of RA, are now rather explained as bi-products of inflammatory activity, whereas anti-CCP antibodies may be more specific for RA [20]. Although the immunity towards citrullinated peptides are thought to contribute towards the development of RA resulting in a distinct subset of anti-CCP positive RA patients, details of the aetiology triggering citrullination are still missing [20,21].

Synovial inflammation is the hallmark of RA and this process is perpetuated by activated B and T cells interacting with macrophage mediated cytokine release [21]. Tumour necrosis factor alpha (TNF- α) is considered a key player in cartilage destruction and bone resorption [22]. TNF- α is also responsible for the activation of endothelial cells and of other cytokines such as IL-1 and IL-6.

2.1.4 Disease course

RA is described as a progressive disease and without treatment it will cause joint destruction, pain and disability in the majority of patients [7]. A study from 1984 published data on the disease progression of 75 patients who had initially been referred to the clinic for intra-articular corticosteroid injections. At inclusion the mean age (range) was 54.7 (27-79) years, and the disease duration was 11.2 years. After 9 years, 20 patients had died and there was evidence of a significantly reduced functional capacity in the survivors. Of the patients who were of such age as to be eligible for work both in 1973 and 1982, 41% were working at inclusion and only 15% after 9 years [23]. Although dated, this study is still relevant today as it describes the course of RA, prior to the use of MTX and modern biologic agents. Data from the ORAR also published prior to the dawn of TNF- α inhibitor

use in Norway, have similarly concluded that at least 40% of newly diagnosed patients had clinically important changes in health status after 5 years [10].

General mortality rates are increased in patients with RA and a recent meta-analysis has reviewed the available literature [2]. Sokka et al. identified 84 cohorts, after excluding serial publications on the same data set. The median standardized mortality ratio (SMR) was 1.57, ranging from 1.62 in the older cohorts, and 1.54 in the most recent studies. The SMR was slightly lower in inception cohorts, with a median value of 1.28 [2]. CVD was the most frequent cause of death, whereas infections were most increased relative to the general population. Cancers in general were not increased, whereas lymphomas were more frequent in patients with RA.

2.1.5 Treatment of RA

The Norwegian pharmacological practice in treating patients with RA has been documented in 1300 patients treated between 1980 and 1993 [24]. This study found that the median time to start of treatment from disease onset was 3.1 years, and reported on a steep increase in the use of MTX during the study-period [24]. The introduction of MTX in the 1980's did indeed bring about great changes in the treatment of RA, as it succeeded gold compounds, sulphasalazine and hydroxychloroquine to become the number one drug of choice. More than a decade later the introduction of TNF- α inhibitors further enhanced the treatment of RA [25-27]. The current EULAR recommendations for the treatment of early arthritis advocate referral to a specialist within 6 weeks of the first symptoms, and the use of MTX as an anchor drug early in the disease course [28]. Studies such as FIN-RACO, TICORA, COBRA, BEST and CAMERA, speak for an early intensive treatment of RA in order to achieve the greatest efficacy, preserve patient functioning, and minimize joint damage [29-33]. Reversing the pyramid from the earlier doctrine of "start low, go slow", will often entail combination therapy early in the disease course and regular monitoring of disease activity in order to achieve the target of remission.

2.2 Cardiovascular disease

2.2.1 Definitions

2.2.1.1 Cardiovascular disease is defined by the World Health Organization (WHO) as “a disorder of the heart and blood vessel, and includes coronary heart disease (heart attacks), cerebrovascular disease (stroke), raised blood pressure (hypertension), peripheral artery disease, rheumatic heart disease, congenital heart disease and deep vein thrombosis” [34]. In this thesis I have chosen to consider acquired coronary heart disease and hypertension as the main clinical endpoints of the CVD process.

2.2.1.2 Arteriosclerosis is derived from the Greek words for artery (arterio) and stiffening (sclerosis) and literally means stiffening of the arteries [35]. Arteriosclerosis is the major cause of CVD and is sub-classified as three separate lesions, although some of these terms are commonly used as synonyms. **Atherosclerosis** is the most common form of arteriosclerosis, and is characterized by atheromas occurring in large and elastic arteries. **Arteriolo sclerosis**, often seen in hypertension and diabetes, is the stiffening of the arterioles (small arteries with 1-2 layers of smooth muscle). Mönckeberg medial calcific sclerosis is, as the name implies, medial calcification of medium to large arteries. The condition is rarely seen in young individuals [36,37].

2.2.2 Pathology of the vessel wall.

2.2.2.1 Historical view

Rudolph Virchow (1821-1902), often described as the “father of modern pathology” is now credited as being the first to describe atherosclerosis as an essentially inflammatory disease, more than a century before this became the prevailing conception. However, he also recognized that lipids were important constituents of atheromatous lesions, describing a non-inflammatory “fatty metamorphosis” of the arteries as being distinguishable from other areas where “irritation” preceded the “fatty metamorphosis” [38].

The focus would later on sway towards the multifactorial causality of CVD and atherosclerosis. Experiments demonstrating the induction of atherosclerosis in rabbits (and later humans) fed on a cholesterol rich diet were pivotal in identifying cholesterol as the chief offender [39]. A much cited epidemiological study confirmed the clinical implications

of this theory in Japanese men, who by changing diets after migrating to California suffered from increased CVD mortality (and cholesterol levels) [40]. Atherosclerosis was thus for a long period viewed as a degenerative disease, characterised by lipid deposition and fibrosis, its inflammatory component being neglected [38,41,42]. The reduction of lipid levels in the population bore fruit, as mortality rates dropped and the incidence of CVD declined [43,44].

2.2.2.2 Atherosclerosis

Ross published his seminal “Atherosclerosis- an inflammatory disease” in 1999, a paper that became central in the modern understanding of atherosclerosis. He pointed to the important role of macrophages and T-lymphocytes in all types of atherosclerotic lesions from the fatty streak that is common in children, to the plaque verging on a rupture [41]. According to modern understanding, the atherosclerotic process starts with a dysfunctional endothelium which has somehow lost its ability to repel leucocytes and which has become permeable to lipoproteins [41,42,45-48]. Leucocytes are attracted by chemoattractants [42], but the actual attachment of the leucocytes to the intima is brought about by the expression of the vascular adhesion molecule-1 (VCAM-1) [42], and other adhesion molecules that are up regulated by pro-inflammatory cytokines in areas of non-laminar flow, typically at sites of arterial branching. The endothelial dysfunction is proposed to be multi-factorial in origin, with oxidized LDL, cigarette smoking, hypertension and diabetes acting through free radicals, as probable causal candidates [41,42,46,49]. The earliest atherosclerotic lesion is thus considered to be the fatty streak, and some of these may develop into plaques [36,47]. The lesions contain monocytes which ingest the lipoprotein as they develop into lipid laden macrophages (foam cells) and perpetuate the inflammatory state by initializing an inflammatory cascade attracting smooth muscle cells, platelets and more monocytes. Gradually a fibrous cap forms, demarcating the lesion from the lumen, and preventing exposure of the necrotic core developing below to the pro-thrombotic agents in the blood stream. The lesion has now become a plaque containing an extra cellular matrix within which calcification and neo-vascularization occurs [41,47]. The plaque develops in bursts, and plaque disruption can occur due to thinning of the fibrous cap or internal haemorrhage. Matrix metalloproteinases are thought to be key players in plaque disruption, and their release can be stimulated by inflammatory mediators and oxidized lipoproteins [42]

As the inflammatory nature of atherosclerosis again is acknowledged, some pathologists have re-opened the 19th century debate concerning the role of infectious agents in the development of atherosclerosis. The most frequently mentioned suspects are

Cytomegalo virus, Chlamydia pneumoniae (C. pneumoniae) and Helicobacter pylori, but also bacteriae from the oral mucosa have been implicated [50]. Microbial antigens have indeed been identified within atherosclerotic plaques [38], and the agents are proposed to act either through generalized inflammation, by direct endothelial injury due to cross-reactivity of antibodies towards heat shock proteins produced by both microbes and endothelium, or through plaque destabilization [38,45]. The proponents of the “infection theory” will explain the reduction in CVD mortality that has occurred in recent decades as partly being due to the widespread use of antibiotics [38]. However a placebo controlled trial that randomized 4012 patients with stable coronary artery disease to receive placebo or a weekly dose of 600mg Azithromax for a year, was negative with respect to the primary outcome of cardiovascular events and there is as of now no indication for treating CVD with antibiotics [51].

2.2.2.3 Arteriolosclerosis and Mönckeberg medial calcific sclerosis

Arteriolosclerosis is a disease process that according to current classifications affects the arterioles and small arteries [36,37]. It can be divided into a hyperplastic and a hyaline form, both of which may give narrowing of the lumen. The hyaline form may be seen in normotensive patients but is reported to be most advanced in patients with hypertension and diabetes [37]. It is a chief morphological finding in benign nephrosclerosis and is due to build up of plasma components and extra-cellular matrix products. The hyperplastic form is often seen in patients with malignant hypertension and the thickening of the wall is thought to consist of smooth muscle cells and basement membrane. Mönckeberg medial calcific sclerosis is a calcification that affects the media of medium and large size arteries and is associated with kidney disease [36].

Recently the classification of arteriolosclerosis and medial calcific sclerosis has been criticized for not reflecting the true pathology of the stiffening artery and for being a inconsequential mix of anatomical and histopathological conditions that are improperly defined [37]. The authors have suggested a novel classification scheme of:

1. Primary arterial calcification
2. Fibromuscular intimal thickening.

Under this scheme intimal calcification and the medial calcification would be termed as primary arterial calcification, whereas the hyperplastic form of arteriolosclerosis would be

termed fibromuscular intimal thickening to allow for the fact that this may also occur in the arteries [37].

2.2.3 Disease course

The atherosclerotic disease process begins in childhood and progresses throughout life at a rate that is determined by individual gene-environment interactions [47]. The atherosclerotic plaques develop primarily in elastic and large/medium sized arteries [36]. The fatty streak, while considered to be the first lesion, is regularly found in regions where it is unlikely that a plaque will develop. The fibrofatty plaques can develop into vulnerable plaques prone to rupture, but it is only when the plaque encroaches on the blood supply to an organ that the clinical disease becomes apparent [41]. Plaque progression or rupture in the coronary arteries may lead to arterial occlusion and stenosis resulting in angina or myocardial infarction, and plaque embolization from the carotid artery may lead to cerebral infarction. Other possible consequences of atherosclerotic disease are peripheral artery disease with resultant gangrene, mesenteric occlusion, sudden cardiac death or chronic ischemic encephalopathy. An atherosclerotic plaque in the elastic aorta may alternatively result in an aneurysm through mural thrombosis and wall weakening [36].

A clinical manifestation of CVD indicates an increased risk of future major clinical outcomes. The pan-European Euro Heart survey monitored 3031 patients with stable angina for a median of 13 months and recorded the occurrence of myocardial infarction or death. They found that the hazard ratio for the combined outcome doubled if the patient had a history of previous myocardial infarction and with increasing angina symptom severity [52]. Yusuf et al. estimated that a history of hypertension gave an OR of 1.91 (CI) (1.71-2.10) for future myocardial infarction. The population attributable risk, which depends on the prevalence of a risk factor, and the risk associated with it, was 17.9% for hypertension in the future occurrence of myocardial infarction [53].

Hypertension is a consequence of a disturbance in the relationship between cardiac output and total peripheral resistance [36]. The cardiac output depends upon the blood volume and is regulated via sodium homeostasis. Peripheral resistance is under continuous control to ensure adequate, but not excessive tissue perfusion. Vasodilators (e.g. prostaglandins and (nitric oxide) NO) and vasoconstrictors (e.g. angiotensin II) exert opposing forces on the vessel wall in order to meet this requirement. The blood pressure of an individual is largely explained by age, gender, BMI and dietary sodium intake, but

genetically determined variations in renin-angiotension system may explain some of the dispersion [36,54]. Blood pressure is equated by cardiac output multiplied by peripheral vascular resistance, the latter being determined by an array of local, humoral and neuro-endocrine factors that work to alter vascular tone. The main vascular blood pressure regulation occurs at the arteriolar level, but also heart rate and cardiac contraction are important determinants of blood pressure. The kidney is the site of sodium regulation acting through aldosterone secretion (increased reabsorption of sodium from urine), production of prostaglandin and NO, or natriuretic factors which can inhibit sodium reabsorption in the distal tubule [36]. The majority of patients suffering from hypertension have no single identifiable cause. On the contrary; a multifactorial origin is often suspected and the patient is diagnosed with essential hypertension [36].

2.2.4 Epidemiology

CVD is the global number one cause of mortality, and 29% of global deaths are attributed to one or other form of CVD [34]. According to the European Society of Cardiology (ESC) more than 4 million people died in Europe as a direct consequence of CVD in the year 2000 [1]. CVD mortality has fallen steadily from the 1960s in Western Europe and North America, and a reduction of CVD risk has occurred in groups with favourable socio-economic resources in the developing world. The people of Central Europe or Eastern Europe, and individuals in poor socio-economic circumstances have unfortunately not displayed the same reduction in mortality as of yet [34].

CVD is the main cause of death also in Norway, although a decline in mortality rate started here in the mid 1970s [44]. Of the CVDs, it is coronary heart disease and cerebrovascular disease that account for the majority of these deaths [44]. Norway was considered a high-risk country with regard to CVD mortality in 1970's. However, the drop in CVD mortality mentioned above, reclassified Norway as being a country of low CVD mortality, with levels comparable to Greece and Spain [44]. Within Norway the inhabitants of the northern regions and the city of Oslo have the shortest life expectancy, and within the city of Oslo there is a pronounced "east:west" gradient that entails increased CVD mortality and greater prevalence of CVD risk factors in areas of social deprivation [55]. Smoking, physical inactivity in leisure time and prevalence of hypertension were the cardiovascular risk factors that showed the greatest geographical disparity in Oslo, in a study from 2001 [55]. In general, the risk of all CVDs increases with age [34] and until late middle age (the

70s), the risk of IHD is 3-4 times higher in males than in females [44]. Women develop CVDs a decade later than men, and dominate the statistics on CVD hospital admissions in the highest age groups [44].

The triad of smoking, high cholesterol and hypertension are considered the most important risk factors for CVD in a population perspective [34,44,53]. Each factor acts as an effect modifier with other risk factors present, increasing the total CVD risk by many times. Cholesterol is the strongest predictor for coronary artery disease (CAD), whereas hypertension is the strongest risk factor for cerebrovascular disease [44]. The specific risk factors are further discussed under biomarkers.

2.2.5 Prevention and Treatment of CVD

According to the WHO 80% of coronary heart disease and cerebrovascular disease are due to behavioural factors of which an unhealthy diet, physical inactivity and smoking are the most important [34]. The primary prevention of CVD is the subject of a plethora of international and national guidelines, but the advice is very much overlapping. The ESC has defined the characteristics of persons who are at low risk of CVD (Figure 1) [1].

The use of risk calculators is advocated in order to estimate the individual's future risk of CVD or general mortality. The most commonly used risk calculators in primary prevention are the SCORE calculator of the ESC and the Framingham risk calculator. The SCORE calculates the 10-year risk of first fatal atherosclerotic event [1] whereas the Framingham predicts 10 year risk of coronary heart disease (CHD) (angina, myocardial infarction, coronary insufficiency and death from coronary heart disease) [56,57]. Age, sex, systolic blood pressure, total cholesterol and smoking habits are predictors in both calculators, but the Framingham also incorporates details of diabetes and treatment for hypertension [57]. SCORE is not valid for individuals with diabetes and it exists in separate versions for high and low risk countries and for those who prefer using the atherogenic index (total cholesterol/ HDL cholesterol) instead of just total cholesterol [1]. Pharmacological intervention is generally recommended to all individuals with established CVD, in patients with diabetes or target end-organ damage or in asymptomatic individuals when the SCORE 10-year risk of fatal atherosclerotic event is $\geq 5\%$ or the Framingham-10 year risk of CHD is $\geq 20\%$. One of the limitations of both SCORE and the Framingham is that young persons do not reach the predicted risk level that necessitates treatment and that older individuals without elevated CVD risk factors have a predicted risk that dictates

treatment. Recently the Norwegian NORRISK has been published with stratification of the risk level that indicates intervention according to the age of the individual [44]. The guidelines recommend that a person aged between 40 and 49 with a 1 % chance of death from CVD within the next 10 years should receive the same attention as a 50-59 year old with a 5% chance or a 60-69 year old with a 10% chance of CVD death. If lifestyle interventions do not succeed in adequate risk reduction, then the practitioner should consider antihypertensive treatment (if blood pressure >140/90), statins for cholesterol reduction (if total cholesterol >5 or LDL cholesterol >3) and acetylsalicylic acid in cases of greatly increased risk [44]. Putting CVD prevention into perspective, the sobering fact is that the majority of individuals who experience a cardiovascular event would have been classified as being at low or intermediate risk according to CVD risk calculations [43].

2.3 Biomarkers

2.3.1. Definition

Biomarkers are quantifiable biological parameters which serve as indices of healthy or pathological processes, and may reflect response to a pharmacological intervention [58]. New biomarkers are developed as we gain greater understanding of the pathological process leading to a disease, and these can give an indication of the patient's position on the pathway to clinical disease. The use of biomarkers can allow for data collection in large cohorts within a relatively short period of time, and should ideally also be cheap to use and validated across different ethnic groups and in a variety of conditions.

2.3.2 Biomarkers in RA

In the field of rheumatology biomarkers have the potential to help predict which individual is at risk of developing RA, and when arthritis is present, who will suffer from persistent erosive disease, loss of function or, as the worst scenario, death. RA is however a multifactorial disease and the truth will never be wholly captured by a prediction model.

As noted above, patients with RA may have anti-CCP antibodies several years prior to a diagnosis and the presence of SE is associated with an increased risk of anti-CCP positive RA, but the incidence rate of RA is relatively low, and both SE and anti-CCP antibodies may also be present in healthy populations. A recent study concluded that 10% of the general population in Britain carried a susceptibility gene for RA [59]. Consequently, screening the general public for RA susceptibility is not a viable method of identifying

individuals at risk at the moment [21]. The European League Against Rheumatism (EULAR) recommendations for the management of early arthritis identified the presence of IgM or IgA RF, anti-CCP, high erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP) levels, joint swellings and early erosions visible on radiographs as predictors of persistent, erosive disease [28].

Measures of patient functioning have high face validity, as it is easy to appreciate the direct relevance of the questions to patient quality of life. Measures that reflect how a patient feels, functions or survives are thus considered clinical endpoints, and biomarkers may be used as surrogates for these endpoints after extensive validation [58,60,61].

In the following I will very briefly highlight some biomarkers that reflect RA disease activity in the analyses composing this thesis. I will try to focus on how these biomarkers relate to what really matters, i.e.; how the patient feels, functions and survives.

2.3.2.1 Soluble markers of disease activity

Markers of inflammation are cross-sectionally more strongly correlated with other disease centred variables such as radiographically assessed joint erosions, anti-CCP and SE, than with the patient reported outcomes, such as fatigue, function and pain [2]. A previous longitudinal analyses on data from the EURIDISS cohort concluded that ESR levels correlated with later physical function, as reported by the Health assessment Questionnaire (HAQ) [62].

CRP and ESR have been shown to be relatively stable during the disease course in the pre-biologic era, and to predict mortality in patients with RA [63-67]. The responsiveness of markers of inflammation in patients with RA treated with TNF- α inhibition, i.e.; the sensitivity to change, is considered to be low and is surpassed by composite disease activity scores and MRI imaging [68].

Early levels of inflammation predict later joint erosion in patients not treated with TNF- α inhibition [69], and a study from the EURIDISS cohort concluded that an algorithm of female gender, IgM RF, anti-CCP and ESR predict radiographic progression, while the level of anti-CCP was the strongest independent predictor of radiographic progression [70]. In several cohorts the levels of RF and high levels of anti-CCP have also been found to predict risk of mortality in patients with RA [65,71,72].

2.3.2.2 Clinical markers of disease activity

RA disease activity can be captured by an array of clinical disease measures. The swollen joint counts (SJC) and tender joint counts (TJC) are intuitively meaningful. These counts are incorporated into composite indexes which attempt to measure additional aspects of disease activity. The Ritchie score is calculated by summation of the number of tender joints according to the Ritchie protocol [73]. The Disease Activity Score (DAS 28) is calculated by the following equation: $0.56 \sqrt{\text{TJC}} + 0.28 \sqrt{\text{SJC}} + 0.70 \ln \text{ESR} + 0.014 (\text{patient global VAS})$ [74]. TJC and SJC here refer to examination of 28 joints, and visual analogue scale (VAS) to asking the patient to rate their present disease state on a 100 mm VAS. The DAS28 can alternatively be estimated by replacing the ESR with CRP values. The Clinical Disease Activity Index (CDAI) is calculated as the sum of the number of swollen joints + number of tender joints + patient global VAS (in cm) + investigator global VAS (in cm) and can be calculated without performing a blood test or using a calculator. If CRP is available then the Simplified Disease Activity Index (SDAI) can be calculated in a similar uncomplicated manner [75].

Clinical markers of RA disease activity show moderate cross-sectional correlations with health status measured by the Health Assessment Questionnaire (HAQ) in several studies, but appear less closely related to other patient centred measures such as fatigue [74,76-80]. In longitudinal studies clinical markers of RA disease have been shown to be early predictors of later levels of joint destruction, functional decline and mortality [69,71,81].

2.3.2.3 Markers of joint destruction

Radiographic assessment of joint damage is recommended in the management of patients with early arthritis [28]. The most commonly used method of quantifying damage of the joints in RA is the van der Heijde modification of the Sharp score (vdHSS) [82]. This score is calculated based on the semi-quantitative assessment of joint erosions, joint space narrowing and joint sub-luxation.

Joint damage will occur early in the disease course in many patients and is, if present, a strong predictor of the later degree of joint damage [69,80]. Measures of joint damage are also predictors of functional decline and increased mortality, although the strength of association is not very strong, and few studies find that they are independent contributors in prediction models of mortality [2,62,81].

2.3.3 Biomarkers in the prediction of cardiovascular disease.

In the following I will present some of the central biomarkers that reflect the pathological stages preceding clinical CVD. I have chosen to give most attention to the biomarkers that are used as surrogate endpoints or predictors in the individual papers of this thesis.

2.3.3.1 Serological markers of arterial vulnerability

CRP

CRP belongs to the pentraxin family of proteins and its production is mainly mediated by IL-6 [48]. The level of CRP in an individual is partly determined by genetic factors and partly by traditional CVD risk factors such as smoking, diabetes type II and adiposity [46,48,83,84]. In pathological situations such as inflammation, infections or tissue trauma, the CRP level can increase substantially. Increased levels of CRP in the high sensitivity range are associated with a higher risk of CV morbidity and mortality in the general population [85,86]. In recent years several assays which quantify CRP below 3mg/L, in the high sensitivity range, have thus been developed. The lower limit of reliable quantification can be as low as 0.15mg/L in some assays [87].

Whether CRP is a causal factor in atherosclerosis or just a convenient biomarker downstream of the real culprit(s) is still not definitively established [88]. CRP is claimed to have atherothrombotic effects on endothelial cell, platelets, macrophages and on smooth muscle [48,89]. Additionally, CRP has been shown to up regulate the angiotensin type-I receptor that facilitates angiotensin II mediated vascular smooth muscle migration and proliferation [90]. Endothelial cells incubated with purified CRP exhibit a downregulation of NO production, and increased IL-18 levels, encouraging the adhesion of monocytes to the endothelium [89]. Intriguingly, a later study reported the opposite finding of increased NO bioavailability after incubation of blood vessels in purified CRP [91]. The finding was further confirmed in a sub-study showing that endothelial dysfunction follows inflammation, in this case after a *Salmonellae typhi* vaccination in humans, but that it occurs prior to the rise of CRP [91].

In conclusion, the sway of opinion seems to be towards the view that CRP is probably not a causal factor for the development of coronary artery disease [45]. This view has gained support from recent population studies showing that polymorphisms in the gene determining CRP levels were not predictors of CVD disease [83].

NT-proBNP

N-terminal pro-B-type natriuretic peptide (NT-proBNP) is the biologically inactive N-terminal fragment of the active hormone B-type natriuretic peptide (BNP) and is part of a family of natriuretic peptides that cause vasodilation, natriuresis and smooth muscle modulation in response to myocardial stretch [92-94]. Granules were identified in the atrium of the heart in the 1950s, but it was De Bold working on murine hearts in the 1970s, who discovered that atrial extracts caused natriuresis when injected intravenously [92,95]. BNP is produced in both atriums and ventricles in response to myocardial stretch [92,94,96,97]. NT-proBNP and BNP release may however, also be up regulated by proinflammatory cytokines [98]. NT-proBNP is partly metabolized in the renal proximal tubule, and reduced renal function may partially accounts for the age-related increase in NT-proBNP levels in the general population [93,94]. The principal pathway for clearance of BNP and possibly NT-proBNP is however, through endocytosis and degradation in cells along the vascular pathway [92].

The level of NT-proBNP predicts all-cause and cardiac mortality, and morbidity in the general population as well as in cohorts of patients with heart failure, kidney disease and stable coronary heart disease [96,99-102]. Significant heart failure is considered unlikely in an untreated patient with a low-normal NT-proBNP, [103] but due to a limited specificity, diagnoses such as sepsis and kidney failure must also be considered with high levels [104]. A multi-marker approach to the diagnosis of cardiovascular events or death has been investigated, and NT-proBNP was found to be the strongest independent predictor, with regard to mortality, in populations with and without previous CVD [105].

Lipids

Low-density lipoprotein (LDL)-cholesterol is considered to be a major cause of CVD and has thus been identified as the main target for cholesterol-lowering therapy [106]. The central position of lipids in CVD risk estimation is illustrated by the INTERHEART study which found that the variable with the highest population attributable risk was a high ratio of apolipoprotein B: apolipoprotein A, explaining close to 50% of the risk for incident myocardial infarctions (MI) [53].

A longitudinal study analyzing data from 300 000 patient found that assesment of two lipoproteins (either total cholesterol and HDL, or apolipoproteins) was sufficient to predict CVD risk. A 1 standard deviation (SD) increase in non-HDL (Total cholesterol –

HDL cholesterol) translated to a 50% increased hazard ratio (HR) for CHD (the same HR as LDL cholesterol and apolipoprotein B), and a 12% increased risk of ischemic stroke. Triglycerides on the other hands were not independent predictors in models that included non-HDL and HDL levels [107].

According to Bradford Hill, the strongest evidence of causations stems from studies showing that an intervention on a perceived risk factor results in a reduced frequency of the unfavourable event [108]. The causal relationship between lipids and CVD is in this context very well proven. The 4S study showed that long term treatment with simvastatin prevented CHD, and in the following year pravastatin was shown to prevent MI and death from CVD [109,110]. The current doctrine is one of intense lipid lowering, especially for individuals with a proven history of CVD [1,111].

2.3.3.2 Functional makers of arterial vulnerability

Endothelial function

In the coronary arteries the level of functioning NO can be measured through an invasive test. Acetylcholine has a vasoconstricting effect on vascular smooth muscle and an opposing vasodilatory function via endothelium mediated NO release (working through signal transduction on G proteins) [112]. A resultant vasoconstriction upon injection of acetylcholine into the left ascending coronary artery is thus taken as a sign of abnormal endothelium dependent flow reserve. This test has been shown to predict CHD (MI, heart failure and PCI) [113,114].

Celermajer and Deanfield developed the procedure towards a non-invasive and reliable estimation of endothelial function. Flow Mediated Dilatation (FMD) depends upon the increased shear stress flow that is the result of reactive hyperemia achieved by inflating a cuff placed around the proximal forearm to supra-systolic pressure. There is a subsequent local release of NO and dilatation of the brachial artery which can be assessed by an ultrasound device [112]. As a control, the response of the brachial artery to a non-endothelium dependent vasodilator such as sublingual glycerol trinitrate (GTN) is recorded [115,116]. Brachial FMD correlates with the severity of coronary artery disease, and is an independent predictor of coronary artery disease [117], and cardiovascular events in a model that include the Framingham risk score [118].

Other, less validated, methods of assessing endothelial function exist. The change in augmentation index (AIx) that occurs as a consequence of consecutive administrations of salbutamol and nitroglycerin can give an estimate of the endothelium dependent and

independent endothelial function, respectively [116]. Soluble biomarkers such as adhesion molecules can be measured in the circulation, and are associated with the presence of atherosclerosis [119]. These molecules sit at the cross-roads between inflammation and endothelial dysfunction, and the multi-factorial origin makes interpretation of results difficult [112]. Additionally, the principle of reactive hyperemia inducing shear stress is utilised in a number of novel devices, including the Itamar, which gauges change digital blood flow in response to forearm ischemia and quantifies the reactive hyperemia index (RHI) [120]. The role of NO release in digital reactive hyperemia has been proven, and the RHI has been shown not only to correlate with both FMD and coronary endothelial function, but to predict future cardiovascular events [120-123].

Brachial blood pressure

A measurement of systolic and diastolic pressure is arguably the cheapest and most frequently used biomarker to assess CVD risk, and both systolic and diastolic pressures have been targeted in risk reduction [115]. A meta-analysis of 61 prospective observational studies examined hypertension as a predictor in 56 335 cardiovascular deaths [124]. The study found that in patients aged between 40 and 69 and without previous CVD, an increase of 20 mmHg in systolic pressure above 115 mmHg, or an increase of 10 mmHg diastolic pressure above 75 mmHg, doubled the risk of death from IHD and stroke. As a consequence of this, and similar findings from the Framingham cohort, the 2007 ESC guidelines for the management of arterial hypertension categorises a systolic pressure < 120 mmHg and diastolic pressure < 80 mmHg as optimal. Systolic pressures in the range of 120-129 and/or diastolic pressure 80-84 are described as normal, systolic pressure 130-139 and/or diastolic 85-90 as high normal and systolic pressure >140 and/or diastolic > 90 mmHg as hypertension [125].

The ESC guidelines recommend measuring blood pressure using a mercury sphygmomanometer, or a validated non-invasive auscultatory or oscillometric semi-automatic device. The patient should be allowed to rest before the measurements, and the measurements should be repeated several times and on several occasions. One should ensure that the cuff correctly fits the overarm of the patient. A 24-hour blood pressure measurement may provide useful information both when diagnosing hypertension and when assessing the effect of medication [125].

Low diastolic pressure combined with elevated systolic pressure (isolated systolic hypertension) equates with a high pulse pressure (PP) (systolic-diastolic pressure) [125,126]. PP is a measure of stiffness in the arteries but has been found to be a weak predictor of future mortality when derived from the pressure measurements in the brachial artery [124].

Arterial stiffness

Central arterial stiffness can be assessed non-invasively by pulse wave analysis [127,128]. The available apparatus utilizes applanation tonometry to record pressure readings in real time. The probe obtains accurate pressure waveforms as it equalises the circumferential pressure by slightly flattening the artery against a bony base. Two theoretical principles are central in the understanding of pulse wave analyses. Pressure measured in an artery is not only a product of the cardiac output divided by systemic resistance, but also of a reflected pressure wave that arises from bifurcations and other hindrances that the efferent pressure wave encounters. Secondly, that increasing arterial stiffness corresponds to increasing pulse wave velocity (PWV). There is increasing recognition that progression in arterial stiffness will impact on the heart by enhancing wave reflection and thus increasing pressure in the ascending aorta and ventricles [129,130].

Central pressure measurements are estimations of the systolic pressure in the ascending aorta. The peripheral muscular arteries exhibit greater arterial stiffness than the central elastic in young and healthy individuals. This gradient of increasing stiffness translates to a PP amplification of peripheral PP > central PP. The amplification is diminished as central stiffness increases, with age or greater prevalence of risk factor for CVD [131]. A transfer function is applied to the pressure measurements of the wave in the radial artery in order to estimate central systolic pressure and the AIX. The transfer function that is commonly used today has been validated against invasively measured central pressures [132]. Indeed, central systolic pressure derived from sphygmomanometry has been found to correlate with invasively measured aortic pressures [132] and with left ventricular mass index [133]. The transfer function has however been criticised for relying on brachial pressure measurements and alternatives such as calibrating the transfer function to central pressure measurements derived from carotid artery tonometry have been suggested [130,134].

The AIx is an estimation of the augmentation of central pressure that is caused by wave reflection and is defined as the change in pressure between the second and first systolic peaks as a percentage of the pulse pressure [132] (Figure 2). The AIx has been shown to predict cardiovascular events and death in populations of hypertensive and atherosclerotic patients [135-137]

Aortic PWV is a measure of the velocity in the aorta and is currently accepted as the gold standard measure of arterial stiffness. PWV can be derived from knowledge of both the transit-time for the pulse wave travelling from the heart to two sites, and the distance between these sites [127,134]. The carotid-femoral PWV (cfPWV) estimates aortic PWV between the site on the carotid and on the femoral artery, where the pulse is most strongly palpated. The recordings of the foot of the pressure wave at the carotid and femoral artery are gated to an electrocardiogram (ECG) as a measure of transit time. There are several methods of estimating the distance travelled, but frequently the distance between the supra-sternal notch and the measurement site on the carotid artery is subtracted from the distance between the supra-sternal notch and the site on the femoral artery [134]. The cfPWV is an established independent predictor of all-cause and cardiovascular mortality in both populations with and without pathological conditions [136,138-144].

Ankle-brachial index

The Ankle-brachial index (ABI) has been used for many years to assess the severity of peripheral arterial disease, but is also an indicator of generalized atherosclerosis. The convenient methodology is an obvious advantage. The standardized method entails a systolic pressure measurement by a pulse sensor or doppler probe in the posterior tibial and/or dorsalis pedis arteries, and the lowest of the distal pressures then being divided by the brachial systolic pressure [145]. This marker is inversely related to cardiovascular risk factors such as smoking and diabetes and low levels are related to an increases incidence of mortality, MI and stroke [145,146].

2.3.3.3 Structural markers of arterial vulnerability

IMT/plaque

Ultrasonography of the carotid artery can identify preclinical atherosclerosis either as an atherosclerotic plaque or as an increased intima-media thickness (IMT). Carotid IMT evaluation by ultrasonography is considered a valid and reliable method and is usually measured in one, or more, of these six locations; the near and far wall of the internal and

common carotid artery or the bulb region [147,148]. The segment chosen for measurement varies between studies, and this is a possible source of variability [149]. IMT is a significant predictor of MI and stroke, although the correlation to coronary atherosclerosis is not very strong [147,149,150].

The presence of plaques in the carotid artery is a strong sign of an increased risk for cardiovascular events [151,152]. However, no agreement exists regarding the definition of when an intimal thickening becomes a plaque, and the decision will often depend on several criteria. Salonen's classification was based on the most advanced lesion and described intimal-medial thickening if the distance between the intimal and luminal surfaces was $>1\text{mm}$. In this paper, a plaque was defined as a distinct area with mineralization or focal protrusion, and became classified as stenotic if it obstructed more than 20% of the lumen [151]. Other papers have defined plaque as an irregular thickening $\geq 1.5\text{mm}$ [153] which, in the recent ARIC study, must be combined either with an irregular shape or abnormal wall structure in order to qualify as a plaque [152].

Coronary artery calcium

Computer tomography (CT) detects calcification of the coronary arteries, an early and highly specific sign of atherosclerosis [154,155]. The Agatston score is calculated for each patient by summing the number of lesion in the four coronary arteries after each has been multiplied by a density factor for the area [154]. There is however evidence that the coronary coverage score is a better predictor; this is the percentage of arteries with lesions [154,155]. Most information on (coronary artery calcium) CAC score comes from studies using electron beam CT (EBCT), but also a multi-detector CT will generate highly reproducible data. The multi-detector CT does, however, give a larger radiation dose than the EBCT, which in itself can reach a radiation dose equivalent to 20 chest radiographs, approaching the limit where there might be a small but measurable increased risk of cancer [156]. The CAC has been found to predict CV events and to improve the discrimination of models which included of Framingham risk score [157].

2.3.3.4 Multiple biomarkers

Risk scores derived from multiple biomarkers are viable instruments that aid the clinician who wishes to assess the CVD risk of an individual [1]. The Framingham risk calculator was developed from data collected at the 12-year follow-up of the Framingham examination of white middle class Americans [158]. The risk score has been elaborated on and updated

many times but the endpoint of CHD events (defined as angina, MI, coronary insufficiency or CHD death) has remained constant[56,57]. The validity of the risk model has been examined in several studies and has been found to vary between populations [159]. For this reason the European SCORE project was initiated, culminating in a risk model that predicts death from CVD based on data submitted from 12 European countries and including over 205 000 individuals, contributing close to 8000 deaths[160].

2.4 Cardiovascular disease in RA

2.4.1 The epidemiology of CVD in RA

Mortality in general and CVD mortality specifically are both increased in patients with RA [2]. About 40% of the deaths in the cohorts included in the meta-analyses performed by Sokka, were due to CVD, and this was the most frequently attributed cause of death [2]. Kvalvik et al. has reported similar findings in a Norwegian cohort of patients with RA; a SMR of 1.49 and CVD as the dominant cause of death[161]. Aviña-Zubieta et al. identified 24 papers that addressed a CVD outcome in RA, (including cerebrovascular accidents) and calculated a meta-SMR of 1.5 for cardiovascular mortality. The mortality due to cerebrovascular disease was almost equivalent to that of IHD (1.52 vs. 1.59) [3].

The absolute risk of CVD death is highest for elderly males with RA, whereas the relative risk is highest for young females [162,163]. The increased risk is evident already early in the disease course. A study from the English early RA study (ERAS) found an increased mortality within the first seven years of disease, although the authors mention that these patients were included before biological therapies became an option [65]. Patients with inflammatory polyarthritis, including RA, have also been shown to be at a higher risk of being admitted to hospital with CVD within the first seven years after symptom debut [164].

An increased risk of death within 30 days post-MI for patients with RA has been reported by several groups [5,165]. The frequency of unrecognized MI, and congestive heart failure, has by others been found to be increased in patients with RA included in the Rochester cohort [4,166]. Also in this cohort the increased risk is apparent early in the disease course, as the patients with RA were more likely to have suffered an MI in the two years prior to RA diagnosis than the sex and age matched control population [4].

2.4.2 Biomarkers of CVD in patients with RA

In the following I will give an overview of studies, published by the early days of this thesis-work, which concern important biomarkers for CVD in patients with RA.

2.4.2.1 Serological markers of arterial vulnerability

Markers of inflammation

The level of inflammation, measured by ESR, has been found to predict mortality in patients with RA in several studies [65,66,71,167] and has also been linked to the development of heart failure[168]. A longitudinal study from the Norfolk Arthritis Register found that baseline CRP levels predicted future CVD mortality [64]. The CRP levels in patients with RA are also partially predicted by traditional CVD risk factors such as smoking and BMI [84]. Wållberg-Jonsson has reported that the adhesion molecules E-selectin and soluble intracellular adhesion molecule (ICAM) are associated with the presence of plaques and increased IMT, respectively[169], while other studies have suggested that level of cytokines such as IL-6 may be more strongly linked to endothelial dysfunction than the traditional markers of inflammation [170].

NT-proBNP

A pubmed search at the time of planning this thesis did not identify any papers concerning either BNP or NT-proBNP levels in patients with RA.

Lipids

Heterogeneous findings concerning the lipid profiles of patients with RA have been reported [171,172]. An adverse lipid profile has been found in cohorts across a range of disease durations and activity states. In addition, the lipid components have been found to be particularly proatherogenic due to oxidation caused by inflammation [46].

Whether patients with RA have increased or reduced levels of total cholesterol is a question of contention. Early in the disease course, total and LDL cholesterol have been found to be elevated compared to controls [173]. This finding is strengthened by the observation that total cholesterol in blood-donors, who later develop RA, is increased prior to clinical disease debut [174]. Chronic high levels of inflammation have, on the other hand, been shown to correlate with reduced total cholesterol levels (HDL, LDL and VLDL), [175] and some studies have found that patients with RA have lower total cholesterol than

matched controls [176-178]. Several possible mechanisms have been explored. Lipoprotein lipase has been found to be decreased in adipose tissue and skeletal muscle in inflammatory conditions [175]. Additionally, an increase in the production of acute phase proteins by the liver may lead to reduced lipoprotein production [175], and increased activity by the reticuloendothelial system may result in increased clearance of LDL-cholesterol [179]. My impression, however, is that the majority of studies have found that the level of LDL cholesterol to be statistically similar in patients with RA and controls, but that the HDL is reduced in these patients, contributing to a decreased total cholesterol and resulting in an adverse atherogenic index [171,180-182].

An early report on the effect of disease modifying agents on the lipid profiles of patients with RA was published in 1997, concluding that treatment with hydroxychloroquine had a beneficial effect on serum lipids [183]. Later studies showed that both corticosteroids and DMARDs can have a beneficial effect on the lipid profile of patients with RA in that the subsequent increase of HDL improves the atherogenic index [173,184]. Treatment with TNF- α inhibitors have also been found to alter the lipid profile in patients with RA by increasing HDL, and thereby also total cholesterol, although the changes are small and possibly temporary [185-187]

2.4.2.2 Functional markers of arterial vulnerability

Endothelial function

The first study reporting reduced endothelial function in RA was designed as an intervention, concurrently showing that the dysfunction was ameliorated by TNF- α inhibition [188]. The case-control part of the study, including only 10 patients with RA, found significantly reduced endothelium dependent and independent vasodilation in patients with RA, but it was only the endothelium dependent dilatation that improved upon treatment. This last finding has been confirmed by others, although the cohorts studied have all been very small [189,190]. Endothelial dysfunction has been reported early in the RA disease course [188,191], and also in young RA patients without any clinical CVD risk factors [192,193]. Gonzalez-Juanatey et al. have suggested an association between the SE *04 allele and endothelial dysfunction attenuation [194].

No studies dealing with the RHI in patients with RA were found.

Blood pressure

The Nurses' Health Study, relying on patient self-reported data found no increased reporting of hypertension in patients with RA [195], a finding that was confirmed in the Rochester cohort and by the much smaller Alkaabi study which drew on assessments of age and sex matched cohorts[4,196]. However, Del Rincon et al. reported an increased prevalence of systolic hypertension in patients with RA after adjusting for differences in age and sex, and McEntgart et al found slightly increased diastolic pressures [176,197] .

Arterial stiffness and central pressure

AIx and PWV are both increased in patients with RA when compared to population controls [198,199]. Major publications on arterial stiffness, published by June 2010, in RA are summarized in Table 1 and 2. Two papers that were published at the start of this thesis are highlighted in italics [198,199]. AIx and PWV were both reported to be increased in patients with RA in these cross-sectional studies, although the studies were both very small including 14[198] and 8 patients [199] respectively. In 2003 Wong and van Doornum reported on reduced small and large artery elasticity in two small studies (53 and 25 patients with RA respectively), and found an inverse association with measures of inflammation [200] and Sharp score [201]. However, the validity of this computerized version of the Windkessel model has since been questioned [202]. Roman et al. found increased regional stiffness in patients with RA using the arterial stiffness index, Young's modulus and Peterson's elastic modulus, three measures that integrate pressures derived from the sphygmocor with estimations of distension obtained from ultrasonographic examinations of the carotid artery[203].

Ankle-brachial index

Patients with RA were more likely to have a low ankle-brachial index than matched controls RA in two studies [196,204].

2.4.2.3 Structural markers of arterial vulnerability

IMT

IMT has been found to be increased in patients with RA in several case control studies [204-206], although Roman et al. reported lower IMT in patients with RA compared to population controls [207]. The studies that have investigated the association between

disease activity and IMT are heterogeneous in design and findings. Increased IMT is associated with increased disease duration, radiographically assessed joint destruction, and reduced functioning, but not markers of inflammation in the study by Kumeda et al, whereas others have reported an association between markers of inflammation and IMT [208,209]. Del Rincon attributed 11-16% of the variance in IMT to demographic variables, whereas RA manifestations explained 1-6% [208].

Plaque

Patients with RA have a greater prevalence of plaques in the carotid artery compared to population controls [207,210]. Dessein et al. and Del Rincon et al. both report that a combination of traditional CVD risk factors with variables of RA disease activity give the best models for explaining the presence of plaques in cross-sectional studies [153,208]

Coronary arterial calcification

CAC has been found to be increased in male patients with RA and in patients with RA of more than 10 years duration [211,212]. CAC may be associated to the metabolic syndrome, RA disease duration and markers of inflammation [211-214].

3. Aim and research questions

3.1 General aim

The aim of this thesis was to investigate the association between RA disease activity and markers of CVD risk in a cross-sectional and longitudinal perspective. CVD risk profiles were compared between patients with RA and community controls. The utility of NT-proBNP in the prediction of mortality in RA was also investigated.

3.2 Main research questions

- 1. Are markers of CVD risk and RA disease activity cross-sectionally associated?**
- 2. Do biomarkers of CVD risk differ between patients with RA and population controls?**

3. Is there a longitudinal association between RA disease activity and markers of CVD risk?

4. Do levels of NT-proBNP predict general mortality in patients with RA and how does this biomarker compare to known predictors of mortality in RA?

4. Material and Methods

4.1 Populations

The four papers in this thesis draw on data collected in three cohorts established at Diakonhjemmet Hospital, Oslo, Norway.

1. The European Research on Incapacitating Disease and Social Support (EURIDISS) cohort was established in Oslo in 1991 [215] and was, as the title states, initially a multicentre European project investigating social support in RA as a model of chronic disease. Patients with RA of short duration (≤ 4 years) were identified by searching medical records at the Department of Rheumatology at Diakonhjemmet Hospital, Oslo and at Martina Hansen's Hospital in the neighbouring county of Akershus. The inclusion criteria were a diagnoses of RA according to the ACR 1987 criteria [9], disease duration ≤ 4 years, age 20-70 years and a residential address in Oslo or Akershus. The exclusion criterias were Steinbrocker class IV, meaning that the patient was confined to bed or wheelchair, and expected to be lost to follow-up. 326 patients were eligible for inclusion of which 268 patients agreed to participate in the study. 30 patients were subsequently excluded, 21 did not fulfill the 1987 ACR classification criteria, six planned to move out of the Oslo region and three were immigrants with difficulties communicating in Norwegian [216]. 238 patients were included at baseline and living patients were subsequently asked to attend the follow-up visits after 1, 2, 5, 10 and 15 years.

2. The Oslo Rheumatoid Arthritis Register (ORAR) was established in 1994 but is continually updated by searching the hospital records for patients given a diagnosis of arthritis or a related condition. The medical record of the patient in question is then hand searched and patients fulfilling the 1987 ACR criteria are included in the register. The

ORAR has been validated for its completeness for patients aged 20-79 through a postal survey of 10 000 inhabitants of Oslo [11]. The patients in the ORAR have been asked to participate in regular follow-up data collections. From this register, 90 patients who had participated in the 1997 data collection, and who had reported a disease onset between 1993 and 1997, were identified. Of these patients, eight were deceased and the survivors were asked to participate in the 2007 data collection.

3. The community controls cohort has recently been established. Community controls were selected at random by Statistics Norway, but we attempted to match controls to patients in our cohorts in strata by using details of age, sex and residential area. Statistics Norway will not provide details of education or income but the residential area in Oslo can to some extent be used as a surrogate of social class [217]. Oslo is divided into 17 boroughs and each borough is indexed for living conditions [218]. This index is based on the number of residents receiving state benefits including unemployment payments and mortality. For the stratification, details from EURIDISS and ORAR patients asked to participate in the 2007 data collection were pooled with patients participating in an Oslo Ankylosing spondylitis cohort, also located at Diakonhjemmet Hospital. We chose to combine our cohorts for this stratification for financial reasons as it will enable us to also use data from the community cohorts in other comparative studies.

Details of the study cohorts used in each paper are presented in Table 3

4.2 Data collection

In each study, patients who were eligible for inclusion in the project received a letter informing them in detail about the investigations that were to be carried out, and asking them to participate in the data collection. The patient information and consent form were approved by the local ethical committee. The data collection was organized as evening clinics at which the patient moved through a series of “stations”. The stations were manned by appropriate healthcare professionals (nurse, medical students and doctors). For the data collections that were performed specifically for the papers in this thesis (10-year EURIDISS, 15-year ORAR and community controls) we aimed to have stability with regard to personnel, apparatus and localization through all cohorts and over the entire period.

Demographics and utility

Demographic and health status variables were collected by self-reported questionnaires, but the patients could consult medical students hosting the data collection if in doubt on how to answer. Health status was measured by the HAQ, a questionnaire that captures how patient function in several areas of daily life [76], and by the generic Nottingham health profile (NHP) [219].

4.2.1 Biomarkers of RA disease activity

Soluble biomarkers

Details of the analytical procedure for each soluble biomarker are presented in the relevant papers. NT-proBNP, anti-CCP and SE were batch analysed from frozen sera/full blood that had been stored at -70° C from 1992 (paper 1 and paper 3) and 1997 (paper 1 and paper 4). Biomarkers analysed at the 2007 examination were analyzed consecutively, ESR by the Westergren method, CRP and total cholesterol by COBAS 6000, and NT-proBNP by a Modular E 170 device, both by Roche Diagnostics, Basel, Switzerland. Anti-CCP and IgM RF at this data collection were batch analysed from sera that had been frozen for a maximum of three years using the ELISA method (Inova Diagnostics®, San Diego, USA and an in house method respectively) as previously reported [70]

RA disease activity

A trained study-nurse performed clinical examinations in order to determine the number of swollen and tender joints. Joint destruction was measured by radiographic damage of the hands and scored according to vdHSS at each time point. [82]

Markers of CVD risk

Blood pressure (BP) was measured after a 5-minute rest in a supine position using the OMRON M7. Several measurements were made until two measurements differed by ≤ 5 in both systolic and diastolic mmHg and heart rate, and an average was then calculated.

We performed pulse wave analysis assessments using the Sphygmocor apparatus (Atcor Australia). We measured the carotid-femoral cfPWV between the site on the carotid and on the femoral artery where the pulse was most strongly palpated. The recordings of the foot of the pressure wave at the carotid and femoral artery were gated to an ECG of cardiac activity as a measure of time. Several recordings were made in each patient (median 5, range 1-20 for AIx, and median 3, range 1-10 for PWV). For the AIx analysis our primary

objective was to obtain recordings with an acceptable wave form and an operator index >85 . The operator index is the apparatus internal control index that is calculated on the basis of sufficient pulse height and minimal pulse wave variation [220]. In cases failing this threshold we accepted three measurements with an index >65 or, in a few very difficult cases (3% of the total number of recordings), measurements with an index >50 and an acceptable waveform. The recordings considered to have the highest quality according to pre-determined requirements were selected for further analyses [220]. Patients suffering from atrial fibrillations were excluded from the analysis. Based on prior studies, the patients were requested to abstain from food, drinks (except for water) and smoking for at least 3 hours prior to examination [127].

The RHI, also called the digital hyperaemic response was measured using the Itamar apparatus® [120]. The patients were asked to recline on a bed in a comfortable position and probes were attached to bilateral index fingers. A sphygmo-manometer cuff was wrapped around an upper arm. After a 5 minute recording of the digital pulse, the cuff was inflated to 200mmHg and the recording examined for any sign of pulsatile activity on the occluded side, in which case the cuff was further inflated, although not above 300mmHg. After 5 minutes occlusion the cuff was released and a further 5 minutes of recording was made during which the hyperaemic phase occurred. The RHI was calculated by the software installed on the Itamar.

B-mode ultrasonographic examinations were performed on bilateral common carotid arteries using a GE Vivid 7 ultrasound scanner (GE Vingmed Ultrasound, Horten, Norway) with a 12 Mhz probe (9-14) linear matrix array transducer. The images were analysed off-line from jpg images. The intima-media thickness (IMT) of a 5mm long section of the far wall, 10 mm proximal to the carotid bulb, was obtained by the AMS analysis software (Artery Measurement System, Thomas Gustavsson, Gothenburg, Sweden). Each 5 mm section generated numerous calculations indicated by mean and median values. The latter were used when comparing study groups.

The Framingham 10-year risk of coronary heart disease and the SCORE 10-year risk of CVD related death were both calculated using standardized risk calculators [1,56]

4.3 Statistical analyses

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) 14-17

4.3.1 Examination of data

Continuous variables were plotted for normality. Skewness is a measure of the symmetry of a variable, while the degree of kurtosis estimates how similar the distribution is to a Gaussian distribution [221]. A skewness and kurtosis below 1.5 was considered acceptable for analyses requiring normal distribution of data. In case of non-normally distributed data we performed a transformation to the logarithm of the 10th base (paper 1) or to the natural logarithm (paper 2-4). Variables that could have a score of 0 were difficult to transform, and were therefore dichotomized at the median (e.g. HAQ scores paper 4).

4.3.2 Bivariate examinations

All four papers include a table of bivariate comparisons of crude data. For these analyses cross-tabulation with χ^2 statistics were used for counts, while student t-test or Mann-Whitney U test was used for data with normal and non-normal distributions as appropriate.

4.3.3. Examinations of associations

In all models the correlations between variables were investigated using Pearson's and Spearman's coefficients for normal and non-normally distributed data, respectively. Paper 1 and paper 2 investigated the cross-sectional associations between markers of CVD risk and measures of RA disease activity.. For these analyses we used univariate linear regression with adjustments for age and sex as a first step of the regression analyses. The equation of the linear regression analyses is: $Y = a + \beta X$. This equation gives an estimation of Y for any X, by fitting a straight line in which "a" is the intercept and β is the slope [222].

Variables that showed an association with the dependent variable at the pre-specified level of significance (usually $p \leq 0.1$) were included in multivariate linear regression models, and variables were removed according to the level of significance until all variables included in the model were significant at the level of $p < 0.05$. As age and sex were perceived to be of fundamental importance they were included in all models regardless of the level of significance. The validity of the final models was verified in a number of ways. Cooks distance was plotted, and the stability of the model was ensured after removal of outliers. Previously excluded variables were consecutively entered into the model to examine for additional confounding.

In paper 1 data from several time-points were used in a mixed model repeated analysis. This approach controls for multiple testing of the same patient in the calculation of the confidence intervals. Another advantage of the mixed model repeated analysis is that it

is able to tackle missing data at one time-point, without removing the patient from the model estimation [223].

4.3.4 Prediction models

In papers 2, 3 and 4 we have performed analyses where the dependent variables were dichotomized and we therefore used logistic regression analyses for these analyses. The equation for the logistic regression analyses is $\log \text{ odds of outcome} = (\beta_0 + x_1 + \beta_2 x_2)$, where the β is the regression coefficient of the associated exposure (x). β_0 is the log odds of the group that has not been exposed to x ($x=0$). Through the logit function the log odds of an outcome through the regression analyses can be transformed into an estimation of the probability of that outcome [222]. The multivariate logistical regression model was constructed in the same manner as the multivariate linear model. The validity of the final model was verified by checking for confounders among previously excluded variables and by ensuring that the model remained stable after the removal of outliers.

Individual risk prediction was estimated by calculating the sensitivity, the specificity and the accuracy of the model. These are defined by the following formulae.

Sensitivity = cases correctly identified/ number of cases

Specificity = non-cases correctly identified/number of non-cases

Accuracy = number of cases correctly identified + number of non-cases correctly identified / number of true positive + false positives + true negatives + false negatives [224].

The ability of the model to discriminate for the outcome of interest can be examined by a receiver operating characteristic (ROC) curve. This graph plots the sensitivity of the model against 1-specificity. A large area under the curve (AUC) indicates that the model shows good discrimination, and the AUC must be >0.5 if the model is to be considered better at predicting the outcome of interest than tossing a coin [224].

4.3.5 Statistical assessments of biomarker validity

The repeatability, i.e. the agreement between measurements repeated under identical conditions, of the biomarkers for CVD risk was assessed by calculating the intra-reader correlation coefficient. The reproducibility of the assessments was assessed by reliability calculations. The agreement between measurements under slightly differing conditions was assessed by calculating the inter-reader correlation coefficient [222,225]. In both cases the variance in results between patients is divided by the sum of the variance between patients plus the variance in the difference between readings.

The number of community controls needed in order to achieve 80% power for detecting clinically significant difference in several measurements at the 5% level of significance was calculated prior to asking participants. (Power is here defined as the probability that a study detects a statistically significant difference of a given magnitude between samples [225]).

4.4. Legal and ethical considerations

The patients and community controls were insured through the “The Norwegian System of Compensation to Patients” while on the hospital premises. All studies were conducted in accordance with the Declaration of Helsinki on ethics in medical research [226]. The research protocol at baseline and on each subsequent data collection was approved by the Regional Committee for medical research ethics. The Norwegian Data inspectorate licensed the storage of research data in our institution, and permission to store biological material was granted by the Directorate of Health. All patients were informed of the study protocol in detail, and signed forms of consent prior to study participation. All participants were also informed of their right to withdraw from the study at any time.

5. Results

5.1 Paper I

Our objective was to examine the cross-sectional associations between markers of inflammation, RA disease activity, medication used and NT-proBNP levels.

In bivariate analyses, comparing the disease characteristics of patients with normal vs. elevated NT-proBNP, we found that these groups were comparable for all variables, except for CRP which was marginally increased in the group with elevated NT-proBNP. Patients who reported having a CVD at the 10-year follow-up had significantly higher levels of NT-proBNP at baseline and after 10 years. In the cross-sectional linear regression analyses of baseline and 10-year follow-up data, we found that CRP was a significant predictor of NT-proBNP levels in the multivariate models. At the 10-year follow-up, creatinine levels and disease duration were also associated with increasing NT-proBNP levels.

We performed a mixed model repeated analyses adjusting for age and sex. In the univariate adjusted analyses several markers of disease activity were associated with NT-

proBNP levels, but again in the final model only the presence of CVD and CRP remained significant. An increase of 10 years of age resulted in an increase of 25 % in NT-proBNP, while an increase of 10 mg/L CRP corresponded to an increase of 9% in NT proBNP levels. A longitudinal time variable was included in the model, and we found a significant trend of increasing NT-proBNP levels between baseline and the 10 year follow-up in the final model.

5.2 Paper II

In paper 2 we compared levels of CVD risk markers between patients with clinically active RA and RA in remission, and compared both these groups to community controls. Secondly, we compared CVD risk between patients with RA categorized according to anti-CCP status, level of joint destruction and presence of extra-articular manifestations.

The cohort consisted of 113 patients with RA and 86 community controls, all aged between 30 and 70 years. 82 patients were judged to have active RA (CDAI >2.8), whereas 31 were in remission (CDAI ≤2.8). The community controls were significantly younger than patients in both RA groups, and were more often males than the patients with active RA. Patients with active disease had lower total cholesterol but higher CRP, lnNT-proBNP, brachial systolic pressure, AIx and central systolic pressure when compared to patients in remission and community controls in analyses adjusted for differences in age and sex. Additionally, RHI and lnPWV levels were both less favourable in patients with active RA than in patients in remission.

Constructing a multivariate logistic regression model with active RA vs. RA in remission as the dependent variable, we found that some of the CVD risk markers, (RHI, lnPWV and AIx) were independently associated with having active RA. Patients with active RA were differentiated from those in remission with 96% sensitivity in age and sex adjusted models. The findings were verified by constructing a “case-control” cohort of 30 patients with active RA matched with a patient in remission of the same age and sex. Again we found that patients with active RA had higher lnPWV and AIx and lower total cholesterol and RHI when compared to patients in remission. Categorization of patients according to anti-CCP status, presence of extra-articular manifestation or level of joint destruction gave less consistent differentiations of CVD risk markers.

5.3 Paper III

We examined the longitudinal impact of early inflammatory (RA) disease activity on measures of arterial stiffness in paper 3. At the 15-year follow-up of the EURIDISS cohort we recorded AIx and PWV in the participants. One hundred and two patients had acceptable recordings of AIx and 98 of PWV. The patients participating in the follow-up were younger, had less inflammation and better function at baseline than non-participants.

In logistic regression analyses elevated baseline CRP predicted increased AIx and PWV (dependent variables in separate models) at the 15-year assessment in both the univariate and multivariate models. Current use of cholesterol-lowering drugs was an independent predictor of increased PWV (β (CI) 6.55 (1.12-34.44) $p=0.045$). In linear regression analyses patients with elevated baseline CRP had significantly higher AIx (β (CI) 2.67 (0.06-5.31) $p=0.045$) and lnPWV (dependent variables in separate models) after 15 years (β (CI) 0.08 (0.01-0.14) $p=0.02$) after adjustments for age, sex and mean arterial pressure. There was a trend of increasing AIx and PWV across quartiles of baseline CRP. These trends were however not significant ($p=0.31$) for AIx, nor for PWV ($p=0.12$).

5.4 Paper IV

In paper 4 we examined the incremental value of NT-proBNP in the prediction of mortality in RA across a panel of established and potentially novel risk factors. Established predictors for all-cause mortality in patients with RA are age, male sex, physical function, comorbidities and rheumatoid factor [2]. We chose to use the 1997 examination of the EURIDISS cohort as baseline, because some information on CVDs had been collected at this time-point. Of the 182 patients, 31 had died during the ten-year follow-up. As expected, the patients who had died were older, more often male, had a higher frequency of CVD-related co-morbidities and poorer functional status (HAQ and NHP) at baseline. They also had higher baseline levels of DAS28, and NT-proBNP, CRP and ESR levels were borderline significantly increased in the patients who died in the follow-up period.

Predictors of 10-year mortality were identified in univariate and multivariate logistical regression analyses. All models were adjusted for age and sex. We chose to examine how separate models performed by comparing prediction on an individual level. The number of patients included in each model varied due to missing data. In the demographic model homozygous SE adjusted for age and sex correctly predicted 8 of 29 deaths. Amongst the variables of disease activity only DAS28 was a significant predictor

with a prediction of 10 out of 30 deaths. None of the co-morbidities were significant predictors at $p \leq 0.05$, but a history of MI approached this level. The dichotomized variables reflecting health status; NHP and HAQ, were both significant in the adjusted analyses, but NHP was surpassed by HAQ in a multivariate “health status model” and lost significance. Elevated NHP adjusted for age and sex predicted 7 out of 27 deaths, whereas higher HAQ score predicted 16 out of 30. NT-proBNP was found to be an independent predictor in the “biomarker model” predicting 12 of 23 deaths.

The final model included DAS28 and NT-proBNP and predicted 14 of 21 deaths and had thus a better sensitivity than the “health status model”. The receiver operator characteristics curves comparing the final model with the health status model showed that the combination of DAS28 and NT-proBNP had the greatest area under the curve although the CI were overlapping and the difference was thus not significant.

5.5 Additional results- Biomarker validity

The validity of biomarkers assessed depends upon the accuracy and precision of the measurements. The repeatability and reliability of the biomarkers used in the 15-year study were explored in the following ways:

RHI: The test-retest reliability (repeatability) of the RHI measurements was assessed by examining 19 colleagues, (both male and female, aged 22-62) twice with a minimum 24 hour period between the examinations. The average intra-reader correlation coefficient for two examiners was 0.84 (0.57-0.94).

PWA: The reliability of PWA was partially verified by asking an experienced examiner to repeat the PWA examination in 19 of the included patients. The inter-reader correlation coefficient was 0.75 (0.51-0.90) for PWV and 0.96 (0.89-0.98) for the AIx. The PWA data-selection procedure was shown to be highly repeatable when the author re-examined the original analysis two months after the first data-selection. New selections were made for 20 patients, based on the same criteria, and the agreement between the first and the second selection was 0.94 (0.88-0.97) for PWV and 1.00 (0.99-1.00) for AIx.

IMT: The IMT measurements were made off-line using the AMS analysis software (Artery Measurement System, Thomas Gustavsson, Sweden). The method was found to be reproducible by comparing the measurements made by two examiners on images where the region of interest had been agreed upon. The inter-reader correlation coefficient for the

median IMT measurements was 0.99 (0.98-1.00). A Bland-Altman plot of the reproducibility of the measurements is presented in Figure 3.

6. Discussion

6.1 Methodology

The following section is a critical appraisal of the validity of our methods.

6.1.1. Patient selection

The four papers included in this thesis present analyses of data collected from patients in the EURIDISS and ORAR cohorts. All patient data originate from patients examined at baseline and follow-up visits of these cohorts, and it is necessary to consider whether these patients are generally representative of patients with RA.

The external validity of the patients in the EURIDISS and ORAR cohorts can be assessed by comparing their disease characteristics to other cohorts [225]. The prospective Lund observational cohort was established in the late 1980s and included 183 patients with an average of 11 months symptom duration at baseline [227]. Patients were primarily recruited through primary care. The cohort was similar to the EURIDISS cohort with regard to average age at baseline, but included fewer women (64% vs. 74%). The major difference between these cohorts is apparent at the 10 year follow-ups when 74% of the Lund patients were RF positive compared to only 50% of patients in the EURIDISS cohort (IgM RF and/or IgARF). However, patients from all three cohorts (Lund, ORAR and EURIDISS) who had disease onset during roughly the same period were comparable with regard to health status (HAQ 1.1, 0.97 and 0.91 respectively) [227,228].

The observed yearly all-cause mortality in EURIDISS cohort in the period 1997 to 2007 was 1.7% which, although somewhat lower than the mortality reported in comparable cohorts [161] is within the confidence intervals reported in other studies [229]. The ORAR cohort is not readily compared to the EURIDISS cohort due to the differences in disease duration, but in the present data collection there were no statistical differences between patients in each cohort with regard to mean age, proportion of females, patients with IgM RF >25 U/ml, or mean current level of DAS28. The ORAR cohort has been extensively

validated for its completeness which is assumed to be 85% [11]. The high level of participation in the cohort vouches for the representativity of ORAR.

The RA diagnosis of each patient included in the EURIDISS and ORAR cohorts was verified by a rheumatologist upon inclusion in the cohorts, and all patients were recruited in a hospital setting in the early-mid 1990s. The patients included in our analyses had a disease onset in the period 1988-1997. The current EULAR recommendation on the management of early arthritis recommends that the patient be referred to a rheumatologist within 6 weeks of symptom debut [28]. This recommendation was not in place at the start of this cohort and it is therefore possible that we have selected a group of patients who are at the more afflicted end of the RA spectrum, while patients less afflicted were treated by their general practitioner.

The patients in the EURIDISS and ORAR seem as a group to represent the milder end of the RA disease spectrum, although the great variance in level of inflammation and joint destruction in each cohort makes them very suitable for the identification of predictors. A potential selection bias may have been amplified due to the loss to follow-up which regularly occurs in longitudinal studies.

6.1.2 Selection of community controls

The community controls were asked to participate in the study after they had been identified by Statistics Norway. The community controls were significantly younger and more often male than the RA patients. These differences were largely explained by the fact that we had pooled the RA cohorts with a cohort of patients with ankylosing spondylitis (AS), and the AS patients were younger and more often male. This merge was done to allow for comparative studies of CVD risk in patients with AS at a later stage. Although the combination of two such different patient groups resulted in statistically significant differences in important demographic variables, the stratification did ensure that patients in each stratum had a number of controls to which they could be compared [230].

The response rate of the community controls invited to participate in the study was 43%, while 57% of the surviving members of the EURIDISS and ORAR agreed to participate at the 15- and 10-year follow-up, respectively. It is thus probable that a selection bias has occurred due to the high level of non-participation in the control population. This selection bias may have threatened the internal validity of our findings in paper 2 [225]. Members of the patient cohort may feel a certain loyalty to our hospital after consultations with the nurses and doctors at our department, and may have felt obliged to participate. The

community controls were specifically chosen due to their lack of previous rheumatic disease and had thus no feeling of loyalty to our department.

The media and patient interest groups have informed their readers about the increased risk of CVD afflicting those with RA, and the patients participating in the examination could therefore partially have been motivated by self-interest. However, there are long waiting lists to see a cardiologist and a great awareness CVD risk in the community and our community controls could also have expected personal benefit. We did not exclude individuals with a history of CVD from the community controls and did in fact observe that the RA patients in remission scored better in both the RHI and PWV when compared to the community controls, indicating that the latter group was not particularly healthy. A selection bias towards community control participation motivated by a personal fear of increased CVD risk would put us at risk of making a type II statistical error, i.e. being unable to identify differences between the populations. However, in paper 2 we did find clear statistically significant differences in the distribution of CVD risk markers between patients with active RA, and community controls.

Analysis of the reason for the non-participation of community controls in case-control studies indicated that age is an important factor, with lower rates of participation among young individuals. In general, a decline in the rate of participation in studies have been noted over the last decades across regardless of the age of the participants[231].

6.1.3 Assessments

The CVD risk markers utilized in the data collections were chosen in order to reflect the stages of the CV disease process from endothelial dysfunction to arteriosclerosis and atherosclerosis. We assessed major confounders and ensured that we collected data necessary to calculate composite risk scores such as the Framingham. We considered FMD as a possible measure of endothelial function, but decided instead to use the Itamar because of time constraints. In addition to the data presented here, we have also assessed the prevalence of plaques in the carotid artery and performed echocardiographic examinations. Several other examinations could have yielded interesting data, but additional data on prevalent atherosclerosis, such as by assessing CAC would have been nicely complimentary to our data.

The Sphygmocor device estimated the foot of the pulse wave using intersecting tangents. The Complior (Fukuda Denshi Co., Ltd., Tokyo, Japan) is another popular device for estimating pulse wave velocity. The Complior registers concurrent pulse waves at carotid

and femoral sites using mechano transducers [127]. The software of the Complior then estimates the points of maximal rate of change on the pressure wave forms and uses the correlation between these points to calculate the speed of the pulse wave [232]. A study comparing the Sphygmocor with the Complior found that the latter device estimated significantly higher values of PWV than the Sphygmocor [232].

When choosing measures of RA disease activity and function we were very much influenced by previous data collections, which permitted comparison of disease state between different examinations and time-points. The ACR “Core set of disease activity measures for RA clinical trials” has described the variables that should be assessed in clinical trials, and our data collection was performed in accordance with this consensus [233]. Joint destruction, a measure of cumulative disease activity, was assessed by conventional x-ray. We would clearly have wished to examine the joints of the hand and wrist with ultrasonography and magnetic resonance imaging to better define RA disease remission [234], but again it was considered too time consuming for the participants.

Bone mineral density was measured in participants for later analyses of the association between osteoporosis and CVD. We considered using the apparatus to assess body composition, but decided against prolonging the burden on the patients.

There are several possible sources of biomarker inaccuracy which would threaten the validity of our findings. In planning the studies we acknowledged that inaccuracy was an inherent fact in any study and attempted to ensure that any bias was distributed at random. All participants were examined in the afternoon and all received the same written information. The staff were encouraged to remain at the same “station” each week. All arterial stiffness measurements were performed by a single examiner. In the following I will present some of the factors that may have impacted on the results:.

Preanalytical variability

Measures of endothelial function, blood pressure and PWV display diurnal variations. Whereas endothelial function is reduced in the early morning, the pressure measurements and PWV reach their peak in the evening in patients with essential hypertension [235]. The assessments in the current studies were all performed in the same room and at the same time of day. All patients and controls received the same information regarding the examinations which were to be carried out and were asked to abstain from food, cigarettes and alcohol for three hours prior to the examination. Ideally, the studies should have been carried out after an overnight fast with controls and patients being examined at the same session, but this

ideal situation was not possible due to logistical and feasibility limitations. We used the examination rooms of our out-patient department in the late afternoon, after regular appointment hours, and could not ask our participants to fast all day. The recommendation of at least a 3 hour fast prior to examinations of arterial stiffness was advocated by the expert consensus of arterial stiffness measurements [127], while the RHI is ideally performed after an overnight fast [120]. After observing that the logistics of the data collection worked well, we started to include control subjects in our study

A key determinant of PWV is of course how the distance travelled by the pulse wave during the transit time is estimated. Like many other groups using the Sphygmocor device [236,237], we chose to measure the distance travelled by subtracting the distance from the supra-sternal notch to the carotid site from the distance between the supra-sternal notch and the femoral site. This is the method that is recommended by the manufacturers [220]. However, the majority of survival studies have measured the distance in a straight line between the carotid and femoral sites [136,143,144]. The recent published “Reference Values for Arterial Stiffness Collaboration” chose to convert all distances measured into direct measurements using a validated conversion equation [238]. Weber et al compared values of the PWV calculated from five different methods of wave travel estimation, to invasively measured PWV, and concluded that the subtraction method chosen in our study gave the most accurate estimate. However, all methods showed an acceptable correlation to the invasively measured PWV (Spearman’s R: 0.73-0.77). A comparison of four of these methods revealed a disparity of 16-31% between PWV estimates, although the correlation between methods was very good (Pearson’s R 0.97-0.99) [239]. The method used in our studies gave the lowest PWV of the four methods. Accordingly, the 12 m/s cut-off point for PWV recommended by the ESC/European Society of Hypertension guidelines corresponds to a PWV of 8.3 -10.6 m/s by the methods used in our studies[232,239].

Another possible source of error in the estimation of distance travelled is due to central obesity, which may stretch the tape measure over the abdomen. Callipers can be used to overcome this problem [239]. Even though the BMI of the patients and controls (mean value 26.1 vs. 25.4) was comparable (student t-test $p=0.29$) we cannot rule out that we have overestimated the distance in a few obese patients, and thus overestimated the PWV in these patients.

Ethnicity may be another source of pre-analytical variation [240]. We did not make any formal registration of ethnic origin but observed that the vast majority of individuals in the ORAR and EURIDISS cohort follow-up were of Norwegian origin. Statistics Norway

chose participants for the community controls at random and patients with non-Norwegian sounding names were also asked to participate. However, the individuals who chose to respond positively to the invitation were also mainly of Norwegian origin.

A recent study found that brachial and central blood pressures were reduced in the late follicular phase of the menstrual cycle when the FMD was at its greatest [241]. We were not able to time our examinations according to the menstrual cycle of our female participants but this source of variability should be randomly distributed amongst patients and controls.

Strengths and limitations of the statistical method

It is known that age impacts differentially on blood pressures, PWV and AIx. The large Anglo-Cardiff Collaborative Trial with 4001 participants found that in contrast to the systolic blood pressure which increased steadily with progressive aging, the diastolic pressure peaked at around 50 years and then declined [242]. In the same study, the rate of increase in AIx was similarly shown to decrease after the age of 50 and the linear regression equation describing the association between age and AIx included a negative age squared (age^2) term. The relationship between age and PWV on the other hand included a positive age^2 term, illustrated by an acceleration of the rate of increase across the age groups. These findings are similar to those reported in participants of the Framingham heart study [237].

We have not included polynomial regressions in our analyses, except for an attempt at improved model fitting in paper 2. An alternative approach would be to analyse patients aged < 50 and ≥ 50 years in separate analyses but this was prohibited due to insufficient numbers.

6.2 Main results

6.2.1 The cross-sectional association between markers of RA disease activity and markers of CVD risk (paper 1 and paper 2)

Paper 1 and 2 are both cross-sectional in design and examine the relationship between RA disease activity and markers of CVD risk. In order to understand the significance of our findings, it is necessary not only to consider the possible impact of inflammation on CVD risk markers, but also to explore the relationship between the markers used in the studies. We found significant repeated cross-sectional associations between CRP and levels of NT-proBNP in the EURIDISS cohort (paper 1). CRP remained independently associated to NT-

proBNP levels even after controlling for known cardiovascular risk factors and the presence of self-reported CVD at the 10-year follow-up. These findings have later been replicated by several authors and levels of NT-proBNP have also been shown to be reduced by treatment with TNF- α inhibition [243,244]. In paper 2 we again found that NT-proBNP levels were associated with RA inflammatory disease activity; as patients with active RA had higher levels of NT-proBNP than patients in remission.

The association between NT-proBNP and markers of inflammation may have several potential explanations. A very interesting paper from the field of cellular biology came to our attention after the publication of the first paper. Ma et al showed that exposing murine cardiocytes to inflammatory cytokines such as IL-1 β and TNF- α resulted in a dose-dependent increase in BNP levels [98]. Similar findings are also reported from experiments on canine cardiac fibroblasts, where BNP release was augmented by TNF- α [245]. The association between inflammation and NT-proBNP levels could plausibly also be related to increased arterial stiffness. A finding in paper 2 was that patients with active RA had higher AIX and PWV than patients in remission. PWV is an independent determinant of BNP levels in models that adjust for left ventricular ejection fraction [246] and NT-proBNP levels have also been found to be associated with PWV in the Framingham Heart Study [247]. A more recent study by Schroff et al reported that arterial stiffness was determined by BNP and CRP levels and suggests that the increased cardiac afterload caused by heightened arterial stiffness could be an explanatory factor. The process of arterial stiffening will increase the speed of the reflected pulse wave, resulting in increased central systolic pressure and ventricular hypertrophy, while reducing the blood flow in the coronary arteries during diastole [133,246]. BNP is released as a consequence of stretching the atrial and ventricular wall and acts through natriuresis to reduce the blood volume while modulating ventricular remodelling [92]. Increased levels of inflammatory markers are, as already mentioned, associated with an increased risk of CV morbidity and mortality in the general population and in patient with RA [64-66,83,85], and flares in disease activity have specifically been linked to the initiation of CHF in RA[168].

The process of ageing naturally entails the stiffening of arteries, but several authors also find that inflammation generates an accelerated increase in arterial stiffness [236,248-250]. There are several theories to explain the connection. Vlachopoulos et al found that a vaccination against *Salmonelle typhi* brought about a short-lived increase in PWV. The authors postulated that matrix metalloproteinases, which also increased subsequent to the vaccination, may cause structural and functional changes in the aortic wall thereby

increasing the aortic stiffness [251]. Another possible mechanism involves inflammatory cytokines which have been shown to up-regulate angiotensin type 1 receptors causing vasoconstriction and hypertension which subsequently may lead to arterial stiffness [54,90]. If we accept arterial stiffness to be a result of both structural and muscular properties of the artery wall, then the interactions between inflammatory markers and the arterial wall may have several modes of action. The net result, as presented by the expert consensus on arterial stiffness, is that inflammation may accelerate the process of arterial stiffening by increased vascular collagen formation, calcification and breakdown of elastin [127].

In paper 2, the RHI was reduced in patients with active RA, indicating a diminished endothelial function. The endothelium injury model is indeed often mentioned as a mechanism of arterial stiffness development. An injury to the endothelium heralds the start of intimal thickening, with a decrease in vascular wall contractile elements as smooth muscle cells migrate to the intima, multiply and lay down extra-cellular matrix [36]. In other studies NO has been shown to be an important regulator of arterial stiffness, possibly through the up-regulation of endogenous vasodilators [252-254]. NO release is mediated by the endothelium and may be blighted by inflammation [89,254,255]. However, although some studies have found cross-sectional and longitudinal associations between inflammation and endothelial dysfunction in patients with RA [191,193], this finding is contradicted by others [256]. In the general population CRP levels appear not to be related to the FMD [257], although reactive hyperemia may be related to inflammation [258]

Arterial stiffness was found to be more closely related to both peripheral and central systolic pressure than measures of atherosclerosis in paper 2. PWV, AIx, peripheral and central systolic pressure were all significantly higher in patients with active RA compared to patients in remission. The relationship between arterial stiffness and hypertension displays a complex circular nature. Arterial stiffness is a predictor for the development of hypertension, while high pressures act on the vessel wall to cause increased stiffening [259,260]. The longitudinal Caerphilly study found that the cumulative heart rate x PP product predicted PWV levels later in life, illustrating how repeated stress on the vascular wall could promote increased stiffness [261]. A review by Cecelja and Chowienczyk concluded that age and blood pressure, rather than other classical risk factors for atherosclerosis such as cholesterol, were the principal determinants of cfPWV [260]. However, both AIx and PWV have been shown to be associated with the presence of coronary artery disease in cross-sectional studies on diverse populations, and hypertension is also a powerful predictor of CVD [135,136,138,262]. The relationship between arterial

stiffness and atherosclerosis is currently under debate [263] and while PWV has also been shown to correlate with the amount of atherosclerosis in an autopsy study, the strength of the association was weak [264].

IMT, a measure of atherosclerosis, was not found to be increased in patients with active RA (paper 2), although many other studies have found increased IMT in patients with RA, even within the first year of RA disease activity [265,266]. Indeed, as noted above, IMT and plaque have both been found to be cross-sectionally related to measures of inflammation [208], although a small recent study did not find that IMT was associated to CRP levels in 59 patients with RA [256].

As previously mentioned, a negative correlation has been reported between markers of inflammation and total cholesterol [175], and it has been suggested that the reduced total cholesterol in patients with RA is largely driven by a fall in HDL [171,172]. In the sub-analysis of the Apolipoprotein-related MORTality Risk (AMORIS study), patients with RA were found to have significantly lower total and non-HDL cholesterol than participants without RA[178]. In paper 2 we found lower total cholesterol in patients with active RA compared to RA in remission and community controls. The HDL levels and atherogenic index did not differ significantly between the groups, but HDL was numerically higher in the community controls. The finding of lower total cholesterol in association with active RA may confuse the clinician wishing to prevent CVD in patients with RA. The recently published paper by Peters et al, gives beautiful illustrations of the negative correlations of both total cholesterol and HDL with CRP levels in a cohort of patients with RA. The impact of inflammation appears to have a greater effect on HDL than total cholesterol, resulting in a positive correlation between atherogenic index (total cholesterol/HDL) and CRP. Indeed, the atherogenic index, not total cholesterol, predicted CV events in this study, and the authors argue that the index should be used when assessing CVD risk in patients with RA [267].

In paper 2, patients with active RA did not have higher Framingham risk score compared to patients in remission and community controls. Despite exhibiting higher systolic blood pressures, this group also had lower total cholesterol which will have contributed towards a lower risk score. The European SCORE risk calculator [1] was elevated for the active RA patients when compared to patients in remission, and this could be due to a greater contribution of blood pressure measurements in this estimation or due to the difference in outcome.

The results presented in paper 1 and 2 indicate that achieving disease remission in RA may reduce cardiovascular morbidity. The studies were cross-sectional in design and we are therefore restricted to making predictions regarding future incidence of CVD. However, the biomarkers used in our studies have all been validated as independent predictors of incident CV events. As previously mentioned, treating RA disease activity with a range of medication from hydroxychloroquine to biologics, may have beneficial side-effects on the lipid profile due to the increase in HDL [183,186]. Several studies had reported on the improvement of endothelial function under treatment with TNF- α inhibitors prior to the start of this thesis, and these findings have again been confirmed, albeit in small studies [268], and repeated in patients treated with rituximab, a novel biologics targeting CD20 cells [269]. Indeed, numerous studies have shown attenuated CVD risk as a consequence of TNF- α inhibition in patients with RA. Peters et al have shown that treatment with adalimumab significantly reduced NT-proBNP levels after 16-weeks in 171 patients with RA [244]. Blood pressure was reduced in the group randomized to receive infliximab under the BeSt study [270]. Furthermore, several studies find that TNF- α inhibition will reduce PWV in patients with RA [236,271,272], for a period of up to 56 weeks [273]. Some have also reported improved AIx under treatment with etanercept [274], although this particular finding is disputed [236,272,275]. IMT has also been reported to decrease during TNF- α inhibition [276], while other authors report IMT to be unchanged after a year of treatment [273]. A study by Del Porto et al points in a direction that is very relevant to our findings. They divided patients into 2 groups according to response to TNF- α inhibition, and found that the group with the best response (\geq ACR 20 response maintained during a year) exhibited a reduction in IMT, while the other group did not [277]. This distinction is also made in a study by Dixon et al, which reported a decreased MI rate in patients showing a EULAR moderate or good response on TNF- α inhibition [278].

The use of DMARD, TNF- α inhibitors and prednisolone was restricted to the patient groups in our cohorts. We could therefore not examine these drugs as confounders of the differential CVD risk between patients and controls. Furthermore, channelling bias may entail that patients with high disease activity were more likely to be given certain medications than patients in remission, and we could not adjust for this in the cross-sectional analyses when comparing CVD risk between patients with active RA and RA in remission. Our solution was to examine the use of these different medications as possible confounders of the distribution of CVD risk between patients with active RA and RA in remission.

Arguably the strongest evidence on the benefits of treating RA disease activity comes from studies looking at “hard” clinical end-points such as MI, CV events and mortality. An early study by Choi et al, reported reduced CVD mortality in patients treated with MTX [279], although Landewé et al reported conflicting results [280]. Even earlier, Krause et al reported that response to MTX treatment was associated with a reduced general mortality in patients with RA [281]. More recent studies from Sweden have found reduced CV events and general mortality in patients treated with TNF- α inhibition [282,283]. Provocatively there are indications that treatment with any DMARD [284], over a sufficiently long period [285] reduces CVD risk, although the fact that the medication is continued by the treating physician does suggest that the drug has some beneficial effect on disease activity.

As previously mentioned, the treatment of patients with RA has changed radically over the past decade. The focus is now on early, intensive therapy in order to achieve remission [33]. The recently published recommendations of an international expert committee have conceptualized the new paradigm as “treat to target” (T2T). The “target” in T2T is clinical remission, and the consensus advocates “abrogation” of inflammation as necessary to preserve physical function. In order to achieve and sustain remission, frequent assessments of disease activity using a composite activity score is recommended [286].

6.2.2 A comparison of CVD risk markers in patients with RA and community controls (paper 2)

In paper 2 patients with active RA were found to have higher NT-proBNP, peripheral and central systolic pressure and AIx than community controls, suggesting that the patients with RA had a higher CVD risk. The patient group was older and more often female, and we adjusted for these differences in the regression models. While patients with RA by the very nature of the disease have higher levels of inflammation than community controls, there are many additional reasons for the unfavourable CVD risk status of patients with RA. We explored BMI, smoking habits, co-morbidities and current medication as possible confounders of the final outcome, although it is impossible to adequately adjust for the cumulative exposure to these confounding risk factors in a cross-sectional study.

There were no significant differences in BMI between patients and controls in paper 2. Furthermore, the BMI did not confound the results of our analyses. Two large cohorts have reported that low BMI is associated with increased mortality in this group [287,288]. One of these papers estimated a reduced risk of death of 9% for each unit increase of the

BMI, and reported lowest mortality for patients with a BMI ≥ 30 kg/m² [288]. In studies on patients with CHD, a U-shaped relationship between BMI and risk of mortality has been noted [289]. Low BMI can be a sign of high inflammatory disease activity in patients with RA, but may also indicate co-morbidities that may by them self confer an excess risk of death. Adjusting for disease activity and co-morbidities in this study ameliorated the negative effect of a low BMI on risk of death [288]. Even though our patients had BMI values that were comparable to the controls, current knowledge suggests that they may have had an abnormal body composition, with increase fat mass and reduced muscle volume [290]. It is therefore a limitation that we do not have data on body composition in our study.

The frequency of smokers (ever vs. never) did not differ significantly between patients with RA and controls in our study. Solomon et al reported a greater frequency of past and present smokers in women with RA, whereas Del Rincon et al did not find significant differences between RA patients and controls [176,195]. As previously mentioned, smoking has in recent years been identified as a probable key player in the pathogenesis of RA[21], and knowledge of this fact may have influenced the RA patient's smoking habits. We did not have sufficient data to pin point the time of smoking cessation and decided to treat smoking as "ever vs. never". In light of current literature it is surprising that smoking was not a confounder of the CVD risk markers in our study [291].

NSAIDs are also possible confounders of CVD risk, and NSAID exposure will be related both to the level of RA disease activity and to CVD risk. The detrimental role of NSAID and COXIB medication in the development of CVD is by now well established after the first analyses of data from the APPROVe (Adenomatous Polyp Prevention On Vioxx) trial [292]. Performing a meta-analyses of 121 placebo controlled trials, Kearney et al found that use of both NSAIDs or COXIB was associated with an increased risk of MI [293]. NSAID and COXIBS, with the possible exception of naproxen, are thought to cause thromboembolic events through the cyclo-oxygenase-2 mediated inhibition of prostaglandin I₂, allowing tromboxane-A to act unopposed in causing platelet aggregation and vasoconstriction [294]. Use of NSAIDs at baseline was not associated with increased AIx and PWV after 15 years in paper 3, but we unfortunately did not have reliable data on cumulative use of NSAIDs. In a small, randomized and placebo controlled study Wong et al failed to find any negative effect on endothelial function or AIx following a 2 week course of Indomethacin [295]. Interestingly, a longitudinal study from the NOAR (Norfolk Arthritis Register) found that use of NSAIDs at baseline was a protective factor, reducing

the risk of a future CVD event. The authors suggest that this may be due to a “healthy user” effect, indicating that rheumatologists are assessing the patient’s CVD risk prior to prescribing this medication [296]. Naproxenolone is the first in a new class of anti-inflammatory drugs which are labelled “Cyclooxygenase-Inhibiting Nitric Oxide-Donating”. This drug becomes metabolized into Naproxen and NO, and the hope is that the NO will counteract the hypertensive effect of Naproxen by regulating vascular tone, by increasing mucosal blood flow and mucous production to protect the gastrointestinal tract[297]. The United States Food and Drug Administration (FDA) has requested further trials of long-term follow-up, but a recently published double blind randomized placebo controlled trial in 810 patients with only a 13 week follow-up, does suggest that the drug has a negligible effect on blood pressure, while giving pain relief similar to that of Naproxen [297].

Physical activity is another possible confounder of the outcome and was regrettably not assessed in the 15-year EURIDISS/10-year ORAR data collection. Solomon et al found that women with RA were less physically active than their age and sex matched controls, and that the level of physical activity was related to RA disease activity [5]. Physical activity is related to a favourable CVD risk profile in patients with RA even after adjustments for disease activity and health status [298].

6.2.3 Longitudinal associations between RA disease activity and CVD risk markers (paper 3)

We reported that elevated inflammation early in the RA disease course is independently associated with increased AIx and PWV after 15 years in paper 3. Population studies have found that 10 years aging corresponds to an increase in PWV of 0.48 m/s in a young adult male, and 1.36 m/s in an elderly male [242]. A baseline CRP above the median predicted an increase in later PWV of 1.1 m/s in our study, a level of change that could potentially be clinically significant. As previously mentioned, an association between early inflammation and CV mortality has been identified in patients with RA [64]. Interestingly cumulative inflammation has also been shown to correlate with future AIx in two small studies of patients with long-standing RA [249,299], whereas PWV has been found to be cross-sectionally related to CRP in other studies [271].

Recently published data from the Caerphilly study confirm our findings in a large population drawn cohort of 825 individuals. Mc Eniery et al demonstrated that CRP levels at baseline were important determinants of AIx and PWV measured 20-25 years later. CRP

was also cross-sectionally associated with both PWV and AIX. This paper discusses the possibility of reverse causation to explain the findings whereby the arterial stiffness would be a factor causing elevated inflammation, possibly through damage to the arterial wall caused by the elevated PP that is secondary to increased arterial stiffness [261]. Our findings suggest that the chain of events could be in the opposite direction as inflammation measured in patients with RA is dominated by arthritic inflammatory activity [84], and inflammation caused by irritation of the vascular wall is in this context very minor [46].

If arterial stiffness in patients with a chronic inflammatory disease is partly determined by early and cumulative inflammation during the disease course, then early and aggressively targeted therapy could potentially reduce future CV morbidity and mortality in these patients. Targeting disease activity with disease modifying anti-rheumatic drugs (DMARD) therapy within the first year after an RA diagnosis has indeed been found to reduce IMT and improve the atherogenic index, indicating a reduced risk of future CVD [266]. The recently published ACR/EULAR collaborative RA diagnostic criteria enable the clinician to diagnose RA at an earlier stage in the disease course, by excluding the presence of conventional x-ray erosions from the diagnostic algorithm [300]. Indeed, a patient presenting with multiple synovitis, elevated markers of inflammation and positive serology, will qualify for the RA diagnoses before 6-weeks of disease duration, provided alternative causes of clinical synovitis are considered unlikely [300].

6.2.4 NT-proBNP as a predictor of general mortality in patients with RA (paper 4)

Our results show that NT-proBNP is an independent predictor of 10-year mortality in this cohort of patients with longstanding RA. Established risk factors for RA mortality were also significant predictors in our cohort and the HAQ score, adjusted for age and sex, had the highest single variable discriminatory power. The validation of CV risk markers in patients with RA is important, but to our knowledge few studies have hitherto focused on this [301].

Variables reflecting RA disease activity can be grouped into two clusters, the variables in each cluster being mutually closely correlated. One cluster includes variables that describe patient centred outcomes; these may be derived from patient self-reported questionnaires of which the HAQ score is an example. The other cluster includes measures of RA disease centred outcomes such as ESR, CRP, RF factor and radiographic joint erosions. In general the patient centred outcomes are reported to be better predictors of mortality than the disease centred variables [2], although they, to our knowledge, have not

previously been compared to SE or NT-proBNP. NT-proBNP as a single variable (adjusted for age and sex) had approximately the same predictive ability as the HAQ score. The final model that included DAS28 was superior to other models, although the confidence intervals (CIs) of the ROC curves were not significantly different. A recent study from the CORRONA registry found that the risk of ischemic events in RA was best explained by a model which included variables of RA severity as well as traditional CVD risk factors [302], although variables of RA severity seemed to have a slightly higher C-statistics for predicting the outcome, than traditional CVD risk factors.

NT-proBNP is known to correlate with ventricular function in patients with CHF but is then increased to much higher levels than those seen in populations without clinical CVD. This biomarker has also become an important screening tool detecting pulmonary arterial hypertension in patients with systemic sclerosis [303]. A study by Bibbins-Domingo et al on patients with stable coronary heart disease is intriguing as it demonstrated the independent contribution of NT-proBNP levels to the prediction of both general, and CVD mortality, in models that adjust for echocardiographic data and level of inflammation [304,305].

6.2.5 Biomarker validation

Biomarkers reflecting CVD risk and RA disease activity are integral to the framework of this thesis. Biomarkers have the potential to fulfil many roles, from being predictors, indicators of disease diagnosis or stage, to prognosticating on future events. This is what makes a biomarker so attractive and why there is a continual drive to launch new candidates on the market [61]. Researchers from the field of cardiovascular medicine and rheumatology have sought common inspiration from the work of The National Institutes of Health (NIH) Biomarkers Definition Working group in developing parallel frameworks for biomarker validation [58]. Both frameworks demand that biomarkers employed in clinical medicine must fulfil demands of accuracy, sensitivity to change and display high repeatability and reproducibility [60,61]. Rheumatologists have conceptualized biomarker validation in the OMERACT (Outcome Measures in Rheumatology) filter in the field of rheumatology. The biomarker must satisfy the filters of truth (face, content, constructs and criterion validity), discrimination and feasibility in order to be acceptable [306,307]. Once a biomarker has been validated, it may come under consideration as a surrogate end-point, and potentially the substitute for a specific clinical end-point in a trial [60,61,308]. A formalized surrogacy ranking scheme has also been developed by OMERACT, ranking biomarkers according to

available studies showing a biomarker relationship to clinical target, rating the study design and the statistical strength of the findings [60,309].

Several of the CVD risk markers studied in this thesis have been validated as surrogate end points of clinical CVD disease and some are accepted as surrogate end point in clinical trials by the FDA[310]. A PWV >12m/s is as previously mentioned, accepted as evidence of target organ damage according to the ESC, although as discussed, this level should probably be lower for the Sphygmocor [1,232]. Papers 3 reporting that CRP is a longitudinal predictor of elevated PWV could therefore be viewed as a study contributing to the validation of CRP as a biomarker of target organ damage. However, the study suffers from lack of baseline information on important and probable confounders such as lipids, and the findings must be validated in other longitudinal cohorts. Paper 4 reports that NT-proBNP is a predictor of general mortality in a 10-year follow-up study, and as death is the most highly ranked patients-centred outcome this study would rate highly in the Target domain. There is however again an unfortunate lack of information on important confounders at baseline which would give a lower score for study design.

7. Conclusions

7.1 Main conclusions

On initiating this work we posed several specific research questions. We can now summarize the following answers to the main research questions:

1. Are markers of CVD risk and RA disease activity cross-sectionally associated?

- CRP is cross-sectionally associated with levels of NT-proBNP and remained an independent predictor of NT-proBNP levels after controlling for known cardiovascular risk factors and the presence of self-reported CVD
- CDAI levels indicating active RA disease were associated with significantly higher levels of NT-proBNP, brachial systolic pressure, PWV, AIx and central systolic pressure as well as poorer endothelial function when compared to patients in RA remission, but also with lower cholesterol levels.

2. Do biomarkers of CVD risk differ between patients with RA and community controls?

- Patients with active RA had significantly higher levels of NT-proBNP, brachial systolic pressure, AIx and central systolic pressure, but lower cholesterol when compared to community controls.

3. Is there a longitudinal association between RA disease activity and markers of CVD risk?

- Elevated inflammation, measured by CRP early in the RA disease course, was independently associated with increased AIx and PWV after 15 years.

4. Do levels of NT-proBNP predict general mortality in patients with RA and how does this biomarker compare to known predictors of mortality in RA?

- NT-proBNP was an independent predictor of 10-year mortality in this cohort of patients with longstanding RA. Established risk factors for RA mortality were also significant predictors in our cohort. The HAQ score, adjusted for age and sex, had the highest single variable discriminatory power, but an age and sex model of NT-proBNP and DAS28 scores gave the best prediction.

7.2 Future research opportunities

The data collection that was initiated at the start of this thesis, the 15-year EURIDISS and the 10-year ORAR, and finally the establishment of a community cohort, will hopefully in the future answer other research questions than the ones presented in this thesis.

The data collected should give us the opportunity to validate our CVD risk markers as predictors of CV related mortality within a 5-7 year perspective. All participants have signed consent forms which will allow us to merge the cohort data with data from the Norwegian death register.

We have taken advantage of the skills developed by ourselves and by other staff during the data collections to plan similar examinations in cohorts of patients with ankylosing spondylitis and osteoarthritis. In this work we have liaised closely with several physiotherapists to ensure that physical activity and fitness are surveyed in these patients groups.

7.3 Clinical implications and future perspectives

If we are to effectively prevent cardiovascular events in patients with RA, we must identify RA disease characteristics which signal an increased risk of CVD. Identification of patients at high risk will support an effective individualized strategy of targeted therapy. Our studies indicate that patients with active RA disease qualify for intensified action in order to prevent future CV events. Of the CVD risk markers measured in these papers brachial systolic pressures is probably the marker most amenable to modification. The EULAR recommendations of CVD risk management recommend an annual screening of CVD risk in patients with RA [311].

CVD risk management in patients with RA should be implemented according to the national guidelines [311]. The possibility of pleiotropic treatments may open up new treatment options. Angiotensin-converting enzyme (ACE) inhibitors and statins have been shown to improve the endothelial function of patients with RA [312,313]. The TARA study further reported on the pleiotropic effects of statins by showing a small but significant reduction in RA disease activity in addition to lipid lowering following treatment with atorvastatin [314]. The future could hold new paradigms of CVD risk control during flares in RA disease activity as a matter of course, with a subsequent alleviation in risk control during states of disease remission.

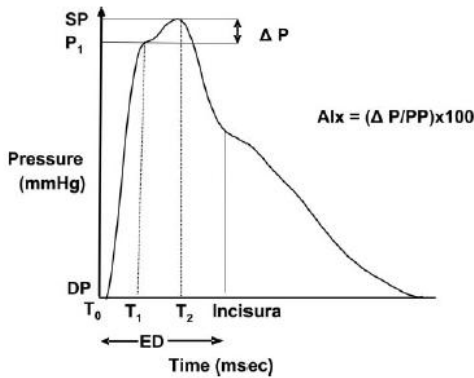
Whether the general practitioner or the rheumatologist should be responsible for assessing and treating the CVD risk factors and instigating the treatment is beyond the scope of this thesis to decide. This study has revealed an unmet need in CVD risk management, a need that is also evident in other contexts [315,316]. The association between active RA disease and increased levels of CVD risk markers discussed in this thesis underscores that the rheumatologist may indirectly modify CVD risk by the aggressive treatment of RA [317]. We hold in our hands weapons which have been proven to reduce CVD risk and their proper use depends upon the vigilance of the rheumatologist.

8. Figures and Tables

Figure 1 Characteristics of the people who tend to stay healthy [1]

1. No smoking
2. Healthy food choices
3. Physical activity; 30 minutes moderate activity daily
4. BMI < 25 kg/m² and avoidance of central obesity
5. Blood pressure <140/90 mmHg
6. Total cholesterol < 5 mmol/L
7. LDL cholesterol < 3 mmol/L
8. Blood glucose < 6 mmol/L

Figure 2 The pulse wave.



Permission to reprint has been granted [318]

T₀; time at start of waveform, T₁; duration from start until first peak of outgoing pressure wave, T₂; duration from start until second peak (reflected pressure wave), ED; ejection duration, SP; Central aortic systolic pressure, DP; central aortic diastolic pressure, P₁; height difference between the minimum pressure and pressure at the first peak (T₁), ΔP; augmentation, (difference between maximal pressure (SP) and pressure at first peak(T₁)), PP₁; pulse pressure, AIx; augmentation index.

Figure 3 A Bland-Altman plot showing reproducibility of IMT measurements.

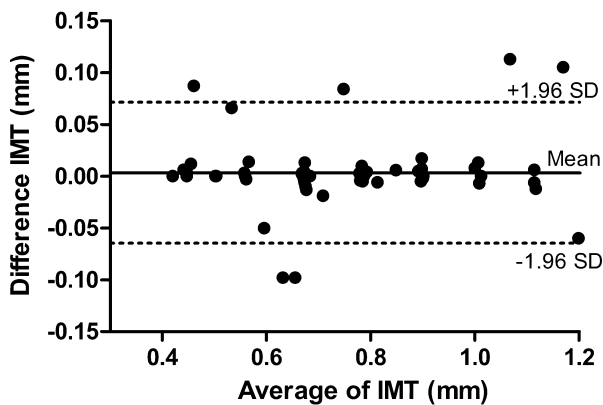


Table 1 Papers concerning the AIx in patients with RA

| Authors | Cohort | Design | Finding |
|-----------------------|---|----------------------------------|---|
| <i>Klocke[198]</i> | <i>14 patients with RA and no CVD, and 14 healthy controls.</i> | <i>Cross-sectional</i> | <i>AIx increased in RA.</i> |
| Mäki-Petäjä[236] | 77 patients with RA and 142 controls. RA patients commenced treatment with etanercept. | Cross-sectional and longitudinal | AIx was not increased in RA and was not altered by treatment |
| Avalos[319] | 117 patients with RA and 65 healthy controls. | Cross-sectional | AIx increased in RA. |
| Pieringer[320] | 36 patients with RA with 36 age and sex matched controls. | Cross-sectional | AIx increased in RA. |
| Wong M[273] | 26 patients with RA who commenced treatment with infliximab. | Longitudinal | AIx unchanged by treatment. |
| Wällberg-Jonsson[299] | 30 patients with RA and 30 controls. Calculated retrospective inflammation score | Cross-sectional | AIx increased in RA and levels correlated with a retrospective inflammation score. |
| Galarraga[274] | 148 patients with RA of which 21 commenced MTX treatment and 26 etanercept treatment. | Cross-sectional and longitudinal | AIx increased in patients with CRP>10 mg/l and was reduced by treatment with etanercept not MTX |
| Crilly[249] | 114 patients with RA and no CVD. Calculated cumulated ESR | Cross-sectional | Increased AIx correlated with cumulated ESR. |
| Angel[275] | 35 patients with RA and other rheumatic disorders commencing treatment with TNF- α inhibitors. | Cross-sectional and longitudinal | AIx unchanged by TNF- α inhibitions. |
| Crilly[321] | 114 patients with RA and no CVD | Cross-sectional | AIx correlated with HAQ and number of damaged joints. |

Table 2 Papers concerning PWV in patients with RA

| Author | Cohort | Design | Finding |
|----------------------|---|----------------------------------|---|
| <i>Yildiz [199]</i> | <i>8 patients with RA</i> | <i>Cross-sectional</i> | <i>PWV increased in RA</i> |
| Mäki-Petäjä[236] | 77 patients with RA and 142 controls. RA patients commenced treatment with Enbrel [®] (etanercept) | Cross-sectional and longitudinal | PWV increased in RA, associated with CRP levels and reduced by treatment. |
| Wong M[273] | 26 patients with RA who commenced treatment with Remicade [®] (infliximab). | Longitudinal | PWV reduced by treatment |
| Stamatelopoulos[322] | 84 patients with RA and 84 controls. | Cross-sectional | PWV increased in RA and correlated with disease duration. |
| Pieringer[323] | 30 women with RA and 30 healthy controls | Cross-sectional | PWV increased in women with RA |
| Angel[275] | 35 patients RA and other rheumatic disorders commencing treatment with TNF- α inhibitors. | Cross-sectional and longitudinal | PWV reduced by treatment |
| Holmes [256] | 59 patients with RA | Cross-sectional | PWV increased in patients with RA but not correlated to CRP levels |

Table 3 showing study cohorts studied in each paper.

| | EURIDISS 1992 | EURIDISS 1997 | EURIDISS 2002 | EURIDISS 2007 | ORAR 2007 | Community controls 2009 |
|---------|------------------|------------------|------------------|------------------|----------------|-------------------------------|
| Paper 1 | x | x | x | | | |
| Paper 2 | | | | x [∞] | x [∞] | x [∞] |
| Paper 3 | x | | | x | | |
| Paper 4 | | x | | x | | |

[∞]Only subjects aged from 30 to 70 years were included in this paper, as per protocol.

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10. Erratum

In paper 2 there is regrettably an error in table 2. The estimated marginal mean of lnNT-proBNP in patients with active RA is 2.40 and not 2.09, the standard deviations and p values for the differences are correct.

11. Papers I-IV

Research article

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The association between disease activity and NT-proBNP in 238 patients with rheumatoid arthritis: a 10-year longitudinal study

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Abstract

Introduction Disease activity in patients with rheumatoid arthritis (RA) is associated with increased cardiovascular morbidity and mortality, of which N-terminal pro-brain natriuretic peptide (NT-proBNP) is a predictor. Our objective was to examine the cross-sectional and longitudinal associations between markers of inflammation, measures of RA disease activity, medication used in the treatment of RA, and NT-proBNP levels (dependent variable).

Methods Two hundred thirty-eight patients with RA of less than 4 years in duration were followed longitudinally with three comprehensive assessments of clinical and radiographic data over a 10-year period. Serum samples were frozen and later batch-analyzed for NT-proBNP levels and other biomarkers. Bivariate, multivariate, and repeated analyses were performed.

Results C-reactive protein (CRP) levels at baseline were cross-sectionally associated with NT-proBNP levels after adjustment for age and gender (r^2 adjusted = 0.23; $P < 0.05$). At the 10-year follow-up, risk factors for cardiovascular disease were recorded. Duration of RA and CRP levels were independently associated with NT-proBNP in the final model that was adjusted for gender, age, and creatinine levels (r^2 adjusted = 0.38; $P < 0.001$). In the longitudinal analyses, which adjusted for age, gender, and time of follow-up, we found that repeated measures of CRP predicted NT-proBNP levels ($P < 0.001$).

Conclusion CRP levels are linearly associated with levels of NT-proBNP in cross-sectional and longitudinal analyses of patients with RA. The independent associations of NT-proBNP levels and markers of disease activity with clinical cardiovascular endpoints need to be further investigated.

Introduction

Patients with rheumatoid arthritis (RA) have a two- to three-fold increase in cardiovascular mortality and morbidity [1,2]. Several studies have reported an association between disease activity and cardiovascular mortality [3-5]. Population-based studies have also shown an increased prevalence of congestive heart failure (CHF) in patients with RA compared with healthy controls and patients with osteoarthritis [6,7]. Measures of disease activity and severity such as C-reactive protein (CRP), disability index, pain, and global severity are

associated with an increased odds ratio for both concurrent and subsequent CHF [6,8].

The level of N-terminal pro-brain natriuretic peptide (NT-proBNP) predicts cardiac mortality and morbidity in the general population as well as in cohorts of patients with heart failure and stable coronary heart disease [9-12]. NT-proBNP, the biologically inactive N-terminal fragment of the active hormone BNP, has a longer half-life than the active hormone and is a viable biomarker of cardiovascular disease (CVD) [13]. In the healthy heart, the main site of BNP production is in the atrial cardiomyocytes, but as the heart fails fetal gene programs are

AIMS1 = Arthritis Impact Measurement Scales 1; anti-CCP = anti-cyclic citrullinated peptide antibody; BMI = body mass index; BNP = brain natriuretic peptide; CHF = congestive heart failure; CI = confidence interval; COX-2 = cyclooxygenase-2; CRP = C-reactive protein; CVD = cardiovascular disease; DAS28 = disease activity score of 28 joints; DMARD = disease-modifying anti-rheumatic drug; ELISA = enzyme-linked immunosorbent assay; HAQ = Stanford Health Assessment Questionnaire; NSAID = non-steroidal anti-inflammatory drug; NT-proBNP = N-terminal pro-brain natriuretic peptide; RA = rheumatoid arthritis; RF = rheumatoid factor; SD = standard deviation; Sharp score = van der Heijde modified Sharp criteria; TNF = tumor necrosis factor.

activated and the ventricular myocardium becomes the main site of BNP production, releasing the peptide in response to stretch or ischemia [9,14].

The associations between clinical and laboratory markers of RA disease activity and levels of NT-proBNP have not been examined. There is also a lack of knowledge on the relationship between exposure to drugs used in the treatment of RA (glucocorticoids, disease-modifying anti-rheumatic drugs [DMARDs], non-steroidal anti-inflammatory drugs [NSAIDs], and cyclooxygenase-2 [COX-2] inhibitors) and NT-proBNP levels in patients with RA. Our objective, therefore, was to examine the cross-sectional and longitudinal associations between markers of inflammation, RA disease activity, medication used, and NT-proBNP levels (dependent variable).

Materials and methods

Patients

We followed 238 patients with RA of less than 4 years in duration longitudinally with comprehensive assessments of clinical and radiographic data at baseline and after 5 and 10 years [15]. The patients had all been recruited to the European Research on Incapacitating Disease and Social Support (EURIDISS) project, which was initially established in 1991. The project has been approved by the Norwegian Regional Committee for Research Ethics. All patients have signed an informed consent form on inclusion and at each follow-up assessment. At baseline, mean (range) age was 51.6 years (23 to 70), mean (standard deviation, SD) disease duration was 2.3 years (1.2), and 73.5% were female. Serum samples were collected from 237 patients at baseline, 126 patients after 5 years, and 145 after 10 years. Eighty-nine patients were lost to follow-up at the 10-year visit (42 declined to participate, 5 had moved out of the area, 35 had died, and 7 gave other reasons not to participate).

Measures of disease activity

Disease activity was measured by joint tenderness, which was assessed at all examinations, and the Ritchie score was calculated [16]. Swollen joint counts were included at the 5- and 10-year assessments; that is, the disease activity score of 28 joints (DAS28) was not available from the baseline visit [17]. Surrogate measures of cumulative disease activity were health status and joint destruction. Health status was measured by the self-administered Stanford Health Assessment Questionnaire (HAQ) and the Arthritis Impact Measurement Scales 1 (AIMS1) [18,19]. Joint destruction was measured by radiographic damage of the hands and scored according to the van der Heijde modified Sharp criteria (referred to below as the Sharp score) [20]. Comorbidities were recorded by a trained study nurse at the 5- and 10-year follow-ups using a checklist modified from AIMS1 concerning the presence of 16 possible comorbidities. One of these items addresses the presence of angina, myocardial infarction, or other cardiac diseases. At the 10-year follow-up, known risk factors of CVD (hypertension,

cholesterol, smoking, and creatinine) were also recorded. The use of NSAIDs, DMARDs, and glucocorticoids was recorded by the study nurse at all assessments (the use of COX-2 inhibitors was recorded only at the 10-year follow-up).

Biomarkers

NT-proBNP was measured using a Modular E 170 device (Roche Diagnostics, Mannheim, Germany). The erythrocyte sedimentation rate was analyzed by the Westergren method at the time of examination, whereas the other biological markers were analyzed from frozen serum or plasma anti-cyclic citrullinated peptide antibody (anti-CCP) by enzyme-linked immunosorbent assay (ELISA) (Inova Diagnostics, San Diego, CA, USA) and CRP by phyCardioPhase high-sensitivity CRP nefelometri (Dade Behring, now part of Siemens AG, Munich, Germany). Rheumatoid factor (RF) IgM was analyzed using the ELISA method [21].

Statistical analyses

NT-proBNP levels were dichotomized according to the national gender- and age-adjusted reference levels modified from Roche Diagnostics guidelines [22] (female: < 50 years of age, NT-proBNP \leq 20 pmol/L; 50 to 70 years of age, \leq 30 pmol/L; > 70 to 80 years of age, \leq 100 pmol/L, and > 80 years of age, \leq 200 pmol/L; male: < 50 years of age, NT-proBNP \leq 10 pmol/L; 50 to 70 years of age, \leq 20 pmol/L; > 70 to 80 years of age, \leq 60 pmol/L, and > 80 years of age, \leq 100 pmol/L). Groups were compared using the χ^2 and Mann-Whitney *U* tests as appropriate.

In the regression analyses, NT-proBNP was entered as a continuous dependent variable and was log-transformed to obtain normality. The presented β -coefficients have been transformed from the log value. All other variables were entered untransformed, and all analyses were adjusted for age and gender. The baseline cross-sectional associations between demographic, disease activity, and severity variables (gender, age, CRP, Sharp score of hands, Ritchie score, HAQ disease duration, RF IgM, anti-CCP, and use of DMARDs, steroids, or NSAIDs) and NT-proBNP levels were explored in bivariate linear regression analyses. Variables that were associated ($P < 0.10$) with NT-proBNP were then entered into a multivariate linear regression model and subsequently removed in a step-wise manner according to levels of significance. These analytic procedures were also applied to the cross-sectional data from the 10-year follow-up examination, which gave the opportunity of controlling not only for cardiovascular risk factors, including body mass index (BMI) and creatinine levels, but also for the presence of self-reported CVD.

Mixed model linear regression was used to examine longitudinal associations [23]. This is a longitudinal linear regression analysis that controls for multiple testing of the same patient by modelling the covariance between the repeated measurements of each individual as a clustered random effect. An

unstructured covariance matrix was used in our analysis assuming that the correlation for each level of within-subject factors is different. The intra-subject variance is then used to calculate the standard error of the regression coefficient. The mixed model procedure deals with missing values by assuming them to be missing at random without removing the individual from the dataset. We investigated the change in NT-proBNP levels over time and examined associations between measures of inflammation, RA disease activity, medication taken, known CVD risk factors, and NT-proBNP (dependent variable). All analyses were performed using SPSS version 14 (SPSS Inc., Chicago, IL, USA). *P* values of less than 0.05 were considered significant.

Results

At baseline, 35 patients had pathological levels of NT-proBNP. Disease characteristics were similar between these subgroups (normal versus elevated NT-proBNP), although the group with elevated NT-proBNP levels had significantly higher CRP levels (Table 1). CRP levels at baseline were significantly associated with NT-proBNP in the linear regression analyses (Table 2). This significant association was also maintained in the multivariate linear regression analyses, which included the interaction between age and gender as an effect modifier. The use of DMARDs, NSAIDs, or glucocorticoids was not significantly associated with NT-proBNP levels. The analysis was repeated with glucocorticoid use dichotomized according to a dosage greater than or less than 7.5 mg (*P* = 0.189).

At the 10-year follow-up examination, age, gender, BMI, creatinine, CRP, and disease duration were significantly associated with NT-proBNP levels (Table 3). Consistent with our baseline analyses, CRP and NT-proBNP levels remained significantly associated in the multivariate analysis. The significant association between disease duration and NT-proBNP levels was

also maintained. The model was also valid if self-reported CVD was entered as a covariate (β coefficient for CVD 1.65, 95% confidence interval [CI] 1.15 to 2.37; *P* = 0.007). The presence of hypertension, smoking status, and prevalent use of glucocorticoids, DMARDs (including subgroups of tumor necrosis factor [TNF] inhibitors and methotrexate users), COX-2 inhibitors, or NSAIDs was not significantly associated with NT-proBNP levels (data not shown).

In the repeated mixed model analysis, several markers of disease activity were associated with NT-proBNP levels, but after multivariate adjustment for time of follow-up, only CRP remained a significant predictor in the final model (Table 4). NT-proBNP levels increased between each follow-up visit and the interactions between age and gender and gender and time of follow-up were significant effect modifiers. The model remained significant if self-reported CVD at the 10-year follow-up was entered as a covariate (β coefficient for CVD 1.48, 95% CI 1.11 to 1.98; *P* = 0.008).

Discussion

NT-proBNP has been shown to be an independent predictor of cardiovascular morbidity and mortality [9-11], and disease activity in RA correlates with mortality, CVD, and CHF [5,6]. Hence, it is of interest to investigate how markers of inflammation and disease activity are related to NT-proBNP in a longitudinal perspective. We found significant cross-sectional and longitudinal associations between CRP and levels of NT-proBNP in this cohort of RA patients with less than 4 years of disease duration at inclusion. CRP remained an independent predictor of NT-proBNP levels after controlling for known cardiovascular risk factors and the presence of self-reported CVD at the 10-year follow-up and in the longitudinal analyses.

Table 1

Baseline characteristics with comparison between patients with pathological versus normal levels of NT-proBNP

| | All patients | NT-proBNP-negative ^a n = 202 | NT-proBNP-positive ^a n = 35 | <i>P</i> value |
|------------------------------|--------------|---|--|----------------|
| Age, years | 51.9 (13.0) | 51.4 (12.9) | 54.3 (13.7) | 0.17 |
| Female gender | 175 (73.5) | 151 (74.8) | 23 (65.7) | 0.26 |
| Disease duration, years | 2.3 (1.2) | 2.2 (1.1) | 2.4 (1.3) | 0.41 |
| RF IgM-positive, \geq 25 | 114 (47.9) | 97 (48.0) | 16 (45.7) | 0.80 |
| Anti-CCP-positive, \geq 25 | 144 (60.5) | 125 (61.9) | 18 (51.4) | 0.24 |
| Sharp score | 6.8 (11.8) | 6.8 (12.3) | 6.5 (8.1) | 0.20 |
| C-reactive protein, mg/L | 9.9 (12.7) | 9.2 (11.8) | 13.9 (15.8) | 0.05 |
| DMARD user | 124 (52.1) | 104 (51.5) | 19 (54.3) | 0.76 |
| NSAID user | 109 (45.8) | 92 (45.5) | 17 (48.6) | 0.74 |
| Glucocorticoid user, oral | 65 (27.2) | 51 (25.2) | 14 (40) | 0.07 |

Values are presented as mean (standard deviation) for continuous variables and as number (percentage) for counts. ^aAge- and gender-adjusted reference levels. anti-CCP, anti-cyclic citrullinated peptide antibody; DMARD, disease-modifying anti-rheumatic drug; NSAID, non-steroidal anti-inflammatory drug; NT-proBNP, N-terminal pro-brain natriuretic peptide; RF, rheumatoid factor; Sharp score, van der Heijde modified Sharp criteria.

Table 2**Cross-sectional associations at baseline between NT-proBNP (dependent variable) and rheumatoid arthritis disease activity**

| | Adjusted linear regression | | Multivariate linear regression | |
|-------------------------------------|----------------------------|----------------|--------------------------------|----------------|
| | β coefficient | <i>P</i> value | β coefficient | <i>P</i> value |
| Age | 1.02 (1.01–1.03) | < 0.001 | 1.009 (1.00–1.02) | 0.04 |
| Male gender | 0.74 (0.59–0.95) | 0.02 | 0.10 (0.04–0.23) | < 0.001 |
| C-reactive protein ^a | 1.01 (1.01–1.02) | 0.002 | 1.01 (1.01–1.02) | 0.001 |
| RF IgM-positive ^{a, b} | 0.96 (0.79–1.17) | 0.67 | | |
| Anti-CCP-positive ^{a, b} | 0.90 (0.74–1.10) | 0.30 | | |
| Glucocorticoid user ^{a, b} | 1.17 (0.94–1.48) | 0.16 | | |
| DMARD user ^{a, b} | 1.04 (0.85–1.26) | 0.71 | | |
| NSAID user ^{a, b} | 0.97 (0.80–1.19) | 0.81 | | |
| Ritchie score ^a | 1.01 (0.99–1.03) | 0.21 | | |
| HAQ ^a | 1.06 (0.90–1.24) | 0.47 | | |
| Sharp score ^a | 1.00 (0.99–1.02) | 0.93 | | |
| Disease duration ^a | 0.99 (0.91–1.08) | 0.82 | | |
| Age \times male gender | | | 1.04 (1.02–1.05) | < 0.001 |
| <i>R</i> ² adjusted | | | 0.23 | < 0.05 |

^aAge- and gender-adjusted; ^bdichotomized variable. anti-CCP, anti-cyclic citrullinated peptide antibody; DMARD, disease-modifying anti-rheumatic drug; HAQ, Stanford Health Assessment Questionnaire; NSAID, non-steroidal anti-inflammatory drug; NT-proBNP, N-terminal pro-brain natriuretic peptide; RF, rheumatoid factor; Ritchie score, Ritchie articular index; Sharp score, van der Heijde modified Sharp criteria.

Our findings are intriguing as the relationship between BNP/NT-proBNP values and the risk of CVD may not be dependent on a threshold value of BNP/NT-proBNP but rather represents a continuum [11,24]. Wang and colleagues [11] found that a single measurement of BNP provided prognostic information in an unselected population of 3,346 persons without heart failure followed for a mean of 5.2 years. An increase of BNP levels by 1 SD was associated with a 77% increased risk of developing heart failure and a 28% increased risk of a first cardiovascular event [11]. BNP has been validated as a marker of CVD in patients with RA. Harney and colleagues [25] found that BNP levels were significantly higher in 26 patients with RA than in controls and correlated with end diastolic volume ($r^2 = 0.83$), end systolic volume ($r^2 = 0.62$), and left ventricular mass ($r^2 = 0.4$). Levels of NT-proBNP have been found to correlate closely with BNP ($r = 0.9$; $P < 0.001$) [24].

Increased levels of inflammatory markers are associated with an increased risk of cardiovascular morbidity and mortality [26,27]. We found that CRP was significantly associated with levels of NT-proBNP at all time points. Flares in inflammatory activity might be better predictors of cardiovascular events than long-term exposure to low-grade inflammation. Indeed, there is evidence that the initiation of CHF in RA patients is preceded by a flare in disease activity [8]. Radiographic progression and the HAQ score are surrogate measures of cumulative disease activity, although we acknowledge that there are

wide inter-individual variations [28–31]. We found no significant association between these measures and NT-proBNP levels in our multivariate longitudinal analyses. Previous reports on the association between joint destruction and CVD in RA have been inconsistent; Wållberg-Jonsson and colleagues [5] demonstrated that early progression of erosions increased the risk of cardiovascular events. Solomon and colleagues [1], on the other hand, did not find that joint erosions were significantly associated with cardiovascular death or myocardial infarction in multivariate analyses. However, we did find disease duration to be significantly associated with NT-proBNP levels at the 10-year cross-sectional analysis. As all patients had a disease duration of 4 years or less at inclusion, the correlation with NT-proBNP levels after 10 years is difficult to interpret. A link between RA disease duration and increased CVD risk can be questioned. In the Rochester cohort, no association was found between risk of heart failure or cardiovascular death and RA disease duration [7,32], nor did Book and colleagues [33] find an association between disease duration and mortality.

To our knowledge, no previous study has examined the association between markers of inflammation in RA and BNP/NT-proBNP. It could be argued that the association between CRP and NT-proBNP identified in our study is a consequence of the inflammatory nature of ventricular remodelling. Patients with RA are not only exposed to systemic inflammation from their

Table 3

Cross-sectional associations at the 10-year follow-up between NT-proBNP (dependent variable) and rheumatoid arthritis disease activity, controlling for cardiovascular risk factors

| | Adjusted linear regression | | Multivariate linear regression | |
|-----------------------------------|----------------------------|---------|--------------------------------|---------|
| | β coefficients | P value | β coefficients | P value |
| Age | 1.04 (1.03–1.05) | < 0.001 | 1.04 (1.03–1.05) | < 0.001 |
| Male gender | 0.92 (0.64–1.31) | 0.64 | 0.76 (0.53–1.04) | 0.09 |
| Body mass index ^a | 0.96 (0.93–0.98) | 0.001 | | |
| Atherogenic index ^a | 0.95 (0.86–1.04) | 0.25 | | |
| Creatinine ^a | 1.02 (1.01–1.02) | 0.001 | 1.01 (1.00–1.02) | 0.04 |
| C-reactive protein ^a | 1.01 (1.0–1.03) | 0.06 | 1.02 (1.00–1.02) | 0.03 |
| RF IgM-positive ^{a, b} | 0.92 (0.72–1.19) | 0.53 | | |
| Anti-CCP-positive ^{a, b} | 1.14 (0.88–1.45) | 0.33 | | |
| Ritchie score ^a | 1.00 (0.99–1.01) | 0.87 | | |
| DAS28 ^a | 1.05 (0.96–1.16) | 0.27 | | |
| HAQ ^a | 1.15 (0.94–1.41) | 0.17 | | |
| Sharp score ^a | 1.002 (1.00–1.01) | 0.17 | | |
| Disease duration ^a | 1.14 (1.02–1.27) | 0.02 | 1.15 (1.03–1.27) | 0.01 |
| R^2 adjusted | | | 0.38 | < 0.001 |

^aAge- and gender-adjusted; ^bdichotomized variable, anti-CCP, anti-cyclic citrullinated peptide antibody; DAS28, disease activity score of 28 joints; HAQ, Stanford Health Assessment Questionnaire; NT-proBNP, N-terminal pro-brain natriuretic peptide; RF, rheumatoid factor; Ritchie score, Ritchie articular index; Sharp score, van der Heijde modified Sharp criteria.

Table 4

Mixed model repeated measure linear regression showing the association between NT-proBNP (dependent variable) and markers of disease activity and treatment

| | Adjusted linear regression (CI) | | Multivariate linear regression (CI) | |
|---------------------------------|---------------------------------|---------|-------------------------------------|---------|
| | β coefficients (CI) | P value | β coefficients (CI) | P value |
| Age | 1.03 (1.02–1.03) | < 0.001 | 1.02 (1.01–1.02) | < 0.001 |
| Male gender | 0.81 (0.64–1.01) | 0.06 | 0.12 (0.05–0.26) | < 0.001 |
| C-reactive protein ^a | 1.01 (1.01–1.02) | < 0.001 | 1.01 (1.01–1.02) | < 0.001 |
| HAQ ^a | 1.12 (1.00–1.27) | 0.06 | | |
| Prednisolone ^{a, b} | 1.20 (1.03–1.41) | 0.02 | | |
| NSAID ^{a, b} | 0.90 (0.79–1.04) | 0.14 | | |
| DMARD ^{a, b} | 0.95 (0.83–1.09) | 0.49 | | |
| Sharp score ^a | 1.01 (1.00–1.01) | 0.001 | | |
| Ritchie score ^a | 1.01 (1.00–1.02) | 0.01 | | |
| Time | 1.04 (1.03–1.06) | < 0.001 | 1.03 (1.02–1.05) | < 0.001 |
| Time \times male gender | | | 1.04 (1.01–1.07) | 0.01 |
| Age \times male gender | | | 1.03 (1.02–1.05) | 0.01 |

^aAge- and gender-adjusted; ^bdichotomized variable. CI, confidence interval; DMARD, disease-modifying anti-rheumatic drug; HAQ, Stanford Health Assessment Questionnaire; NSAID, non-steroidal anti-inflammatory drug; NT-proBNP, N-terminal pro-brain natriuretic peptide; Ritchie score, Ritchie articular index; Sharp score, van der Heijde modified Sharp criteria.

arthritic disease, the failing heart itself may be invaded by peripheral blood mononuclear cells that participate in left ventricular remodelling. These and other inflammatory cells release TNF- α and interleukin-6, thereby increasing CRP levels through upregulation of hepatic synthesis [27].

The strength of the present study is the prospective longitudinal design with regular clinical examinations, blood sampling with subsequent batch analyses, and documentation of radiographic progression. NT-proBNP has been shown to tolerate routine laboratory handling and may be analyzed from frozen sera [10,34]. The validity of our model is supported by the association between NT-proBNP levels and self-reported CVD. Furthermore, in agreement with previous studies, creatinine levels predict NT-proBNP in the final model. NT-proBNP is metabolized in the renal proximal tubule, and reduced renal function partially accounts for the age-related increase in NT-proBNP levels in the general population [14,35]. We found BMI to be inversely correlated with NT-proBNP levels in the adjusted bivariate analyses although it did not reach significance in the multivariate analysis. Other studies have confirmed the inverse relationship between BMI and NT-proBNP levels [36].

This study has several shortcomings. Two hundred thirty-eight patients were included at baseline, and 145 completed the 10-year follow-up. The small scale of this study is a limitation in the analyses. CVD in RA was not a major focus when the cohort was established, and information relating to cardiovascular comorbidity, smoking, concomitant medication, blood pressure, or BMI was not recorded at baseline. The presence of CVD was recorded by a questionnaire at the 10-year follow-up and we did not have the opportunity to verify the diagnosis by searching the medical records. Cause of death for the 35 patients who died during the study was not recorded. Thus, we considered that the data were not sufficiently robust to allow an explorative analysis of how levels of NT-proBNP predict future CVD. However, the patients were requested to report current medication. There was perfect agreement between self-reported CVD and the reported prescription of medication typically used to treat CVD, such as anti-hypertensive drugs, anti-platelet agents, or nitrates, allowing for an internal consistency check.

Conclusion

CRP levels were linearly associated with levels of NT-proBNP in this cross-sectional and longitudinal study of 238 patients with RA. Further studies are needed to elucidate how this biomarker can be implemented in clinical decision making in RA.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SAP developed the study, performed the analyses, and drafted the manuscript. KA participated in the development of the study and in the drafting of the manuscript. SØ participated in the data collection and the drafting of the manuscript. PM gave statistical advice and helped in the drafting of the manuscript. DA conceived the study, participated in its design and development, and helped in the drafting of the manuscript. TKK conceived the study, participated in its design and development, and helped in the drafting of the manuscript. All authors have read and approved the manuscript.

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Remission is the goal for cardiovascular risk management in patients with rheumatoid arthritis: a cross-sectional comparative study

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ABSTRACT

Objectives To compare markers of cardiovascular disease (CVD) risk between patients with rheumatoid arthritis (RA) in an active disease state and those with RA in remission, and to compare both groups with community controls.

Methods 113 patients with RA and 86 community controls were assessed across a panel of biomarkers for CVD. RA in remission was defined as Clinical Disease Activity Index ≤ 2.8 . Community controls were selected at random by Statistics Norway, and controls were matched with patients in the cohorts in strata using details of age, sex and residential area. A panel of biomarkers (N-terminal pro-brain natriuretic peptide (NT-proBNP), total cholesterol, reactive hyperaemia index (RHI), pressure measurements, measures of arterial stiffness and intima-media thickness) were compared between patients with active RA and those with RA in remission. Both groups were compared with controls. In addition, biomarker levels were compared across subgroups based on anticyclic citrullinated peptide status, level of joint destruction and presence of extra-articular manifestations.

Results Patients with active RA had significantly higher levels of NT-proBNP, brachial systolic pressure, augmentation index and central systolic pressure but lower cholesterol than patients in remission and controls. In addition, patients with active RA had significantly higher levels of pulse wave velocity and worse RHI than patients in remission. Comparison across other subgroups gave less consistent differentiations in levels of CVD risk markers.

Conclusion Patients with active RA, but not those in remission, had significantly increased levels of CVD risk markers. These results link inflammatory activity to markers of CVD risk in patients with RA and may indirectly support the notion that remission in RA confers diminished cardiovascular morbidity.

BACKGROUND

Patients with rheumatoid arthritis (RA) have an increased risk of cardiovascular disease (CVD) and a reduced life expectancy.^{1,2} The increased occurrence of CVD is partly explained by an adverse combination of traditional risk factors and factors related to RA disease activity such as inflammation.

The individual risk of CVD can be assessed by biomarkers that reflect many facets of CVD-related pathology and morbidity.³ The pathogenesis of CVD involves endothelial dysfunction, arterial stiffening and atherosclerosis which may ultimately lead to clinical disease, evident as hypertension,

myocardial infarction, stroke or heart failure.^{4,5} A huge array of biomarkers is becoming available to audit the CVD risk at the level of the individual.

The primary aim of the current analyses was to compare levels of CVD risk markers between patients with clinically active RA and those with RA in remission and to compare these two groups with community controls. We also compared CVD risk markers between subgroups of RA patients categorised according to anticyclic citrullinated peptide (anti-CCP) status, level of joint destruction and presence of extra-articular manifestations.

METHODS

One hundred and thirteen patients with RA and 86 community controls aged 30–70 years were assessed across a panel of biomarkers for CVD. The data for the patients with RA were derived from the 15-year follow-up of the EURIDISS cohort ($n=80$)⁶ and the 10-year follow-up of the Oslo RA register ($n=33$).⁷ The patients had been diagnosed according to the 1987 American College of Rheumatology criteria.⁸ The patients were stratified according to the following groups: female versus male, age in decades and residential area. Two hundred community controls were randomly selected by Statistics Norway from the inhabitants of Oslo to match these stratifications. The only exclusion criterion was a history of inflammatory arthritis. Demographic and health status variables were collected by a self-reported questionnaire, but the subjects could request help from attending staff. Body mass index (BMI) was calculated. The patients were requested to abstain from food, drink (except for water) and smoking for at least 3 h prior to examination.

RA disease activity

A trained study nurse blinded to the CVD risk profile of the patients assessed the number of swollen and tender joints (28 joint counts), and disease activity was assessed by the Clinical Disease Activity Index (CDAI) as the sum of the number of swollen joints + number of tender joints + patient global VAS (in cm) + investigator global VAS (in cm).⁹ Patients with a CDAI ≤ 2.8 were considered to be in remission.¹⁰ Joint destruction was measured by radiographic damage of the hands and scored according to the van der Heijde-modified Sharp criteria (vdHSS).¹¹ The presence of extra-articular manifestations was self-reported using a modification of the AIMS 2 questionnaire.¹²

Soluble biomarkers

Biomarkers were examined consecutively: erythrocyte sedimentation rate by the Westergren method, C reactive protein (CRP) and total cholesterol by COBAS 6000 (Roche Diagnostics, Basel, Switzerland), N-terminal pro-brain natriuretic peptide (NT-proBNP) by a Modular E170 device (Roche Diagnostics, Basel Switzerland; coefficient of variation (CV) 7.6–9.7%) and anti-CCP using an ELISA method (Inova Diagnostics, San Diego, California, USA).

Markers of CVD risk

Brachial blood pressure was measured after a 5 min rest in a supine position using the OMRON M7 (Kyoto, Japan). Several measurements were made until two measurements differed by ≤ 5 mm Hg in both systolic and diastolic mm Hg as well as heart rate, and averages were then calculated. We performed pulse wave analysis assessments using the Sphygmocor apparatus (Atcor, West Ryde, Australia). The apparatus uses applanation tonometry, equalising the circumferential pressure by slightly flattening the artery to obtain accurate pressure waveforms. Patients suffering from atrial fibrillations were excluded from the analysis. Several recordings were made in each patient and the recordings considered to have the highest quality according to predetermined requirements were selected for further analyses.¹³

The reactive hyperaemia index (RHI), also called the digital hyperaemic response, was measured using the Itamar apparatus (Caesarea, Israel).¹⁴ Eighteen controls did not participate in this examination for logistical reasons. The patients were asked to recline on a bed in a comfortable position and probes were attached to bilateral index fingers. A sphygmomanometer cuff was wrapped around the upper arm. After a 5 min recording of the digital pulse, the cuff was inflated to 200 mm Hg and the recording examined for any sign of pulsatile activity on the occluded side, in which case the cuff was further inflated, although not above 300 mm Hg. After a 5 min occlusion the cuff was released and a further 5 min of recording was made during which the hyperaemic phase occurred. The RHI was calculated by the software installed on the Itamar.

Pulse wave velocity (PWV) was calculated from knowing both the transit time for the pulse wave travelling from the heart to two sites and the distance between these sites.¹⁵ We chose to estimate the carotid-femoral PWV between the site on the carotid and on the femoral artery where the pulse was most strongly palpated. The recordings of the foot of the pressure wave at the carotid and femoral artery were gated to an ECG as a measure of transit time. The distance between the suprasternal notch and the measurement site on the carotid artery was subtracted from the distance between the suprasternal notch and the site on the femoral artery.

Central systolic pressure and the augmentation index (AIx) were derived by applying a validated transfer system to recordings of the arterial pressure waves at the radial artery. Central systolic pressure is an estimation of the systolic pressure in the ascending aorta. AIx is defined as the change in pressure between the second and first systolic peaks as a percentage of the pulse pressure and was standardised to a heart rate of 75 bpm.¹⁶

B-mode ultrasonographic examinations were performed on bilateral common carotid arteries using a GE Vivid 7 ultrasound scanner (GE Vingmed Ultrasound, Horten, Norway) with a 12 Mhz probe linear matrix array transducer.^{9–14} The images were analysed offline from JPG images as single readings performed by two of the authors (ES and JH). The intima-media

thickness (IMT) of a 5 mm long section of the far wall, 10 mm proximal to the carotid bulb, was obtained by the AMS analysis software (Artery Measurement System, Tomas Gustavsson, Gothenburg, Sweden). Each 5 mm section generated approximately 50 IMT calculations and median values were used when comparing study groups.

The reliability of all biomarkers was tested by repeated examinations in a subset of patients. The inter-reader correlation coefficient was then calculated. For all CVD risk markers the inter-reader correlation coefficient was found to be good (see online supplement for further information).

The Framingham 10-year risk of coronary heart disease and the European Society of Cardiology SCORE 10-year risk of CVD-related death were calculated using standardised risk calculators.^{17 18}

Statistical analyses

Bivariate comparisons were made using the Student *t* test, χ^2 test or Mann–Whitney *U* test as appropriate.

The level of CVD risk markers was compared across the following groups using analysis of covariance (ANCOVA): active RA, RA in remission versus community controls; anti-CCP status positive versus negative; vdsHs joint destruction \leq median versus $>$ median; and presence versus absence of extra-articular manifestations. All analyses were adjusted for age and sex. Skewed variables were log transformed. The validity of the ANCOVA models was explored by examining the following variables as possible confounders in the final model: smoking (now/never), BMI, education, presence of relevant comorbidities (prior myocardial infarction, cerebral insult and diabetes) and current use of CVD-related medication (statins and antihypertensive medication). In addition, the heart rate was explored as a possible confounder of brachial and central systolic pressure and PWV, and height as a confounder of AIx. Disease duration and current RA disease treatments (methotrexate, prednisolone, tumour necrosis factor α (TNF α) inhibitor) were entered into the models which compared patients across categories of RA disease activity. The results were further verified by constructing a case-control cohort matching a patient with active RA to a patient in remission of the same age and sex. In a mixed model procedure we compared levels of CVD risk markers between these groups. A variable denoting each pair was included in the model as a random effect.

A logistic regression model was constructed with active RA versus RA in remission as the dependent variable in order to assess the independent contribution of each CVD risk marker. Individual CVD risk markers were entered consecutively into models that were adjusted for age and sex. CRP was omitted from these analyses at it is a marker of both RA disease activity and CVD risk. Variables that were significant at $p \leq 0.10$ were entered into the multivariate model and removed according to level of significance. Due to multicollinearity, separate models were constructed, each of which only included one of the following variables—brachial systolic pressure, central systolic pressure and AIx—and the model with the greatest Nagelkerke value and accuracy was chosen. Rejected variables were re-entered into the final model to check for possible confounding.

The statistical analyses were performed using SPSS Version 14.

RESULTS

Eighty-two patients had active RA (CDAI > 2.8) and 31 were in remission (CDAI ≤ 2.8). The demographic data are presented

in table 1 and show some differences between the groups, especially for active RA versus the controls. The participation rate of the community controls was 43%, while 57% of the surviving participants of the EURIDISS and Oslo RA register cohorts agreed to participate at the 15- and 10-year follow-up, respectively.

The results of the adjusted comparisons of CVD risk markers (estimated marginal means) are shown in table 2. Compared with patients in remission, those with active disease had higher CRP, NT-proBNP, PWV, brachial systolic pressure, Alx and central systolic pressure, whereas total cholesterol and RHI were lower in patients with active RA ($p<0.05$ for all comparisons). The CVD risk profile in patients with active RA remained significantly disadvantageous when compared with community controls, although the total cholesterol was lowest in the patients with RA. In contrast, patients in remission only had significantly higher CRP levels when compared with the controls, and PWV and RHI levels were more favourable in these 31 patients than

in the controls (table 2). We did not identify any variable that altered the consistency of our findings by confounding several models.

Thirty pairs were matched in the case-control study. The comparisons confirmed the results of lower total cholesterol ($p=0.02$), worse RHI ($p=0.001$), higher CRP ($p=0.02$), PWV ($p=0.01$) and Alx ($p=0.01$) in patients with active RA compared with patients in remission.

The logistic regression analyses with active RA versus RA in remission as the dependent variable found that a model composed of the following variables best differentiated between these patients: RHI (OR 0.15 (95% CI 0.06 to 0.39)), Alx (OR 1.10 (95% CI 1.02 to 1.18)) and PWV (OR 1.72 (95% CI 1.10 to 2.68)), with adjustments for age (OR 0.09 (95% CI 0.84 to 0.98)) and sex (female sex OR 2.98 (95% CI 0.66 to 13.50)). The accuracy of the final model was 84%, sensitivity 96%, while the Nagelkerke value (pseudo R^2)¹⁹ was 0.42. Entering use of antihypertensive medication into the model (OR 11.46 (95% CI 1.76

Table 1 Cross-sectional demographic data compared across groups

| Variable | Active RA (n=82) | RA in remission (n=31) | Population controls (n=86) | p Value (active vs remission) | p Value (active vs control) | p Value (remission vs control) |
|---------------------------|------------------|------------------------|----------------------------|-------------------------------|-----------------------------|--------------------------------|
| Demographic data | | | | | | |
| Age | 56.9 | 58.1 (8.0) | 53.7 (10.5) | 0.54 | 0.05 | 0.02 |
| Female sex | 64 (78.0) | 22 (71.0) | 53 (61.6) | 0.43 | 0.02 | 0.35 |
| Education | 35 (42.7) | 14 (45.2) | 38 (46.3) | 0.81 | 0.64 | 0.91 |
| Smoking | 21 (25.9) | 4 (12.9) | 20 (23.3) | 0.14 | 0.69 | 0.22 |
| BMI | 26.3 (5.6) | 25.0 (3.9) | 25.4 (4.0) | 0.28 | 0.22 | 0.66 |
| Disease duration | 17.1 (2.1) | 16.9 (1.7) | | 0.70 | | |
| Presence of comorbidities | | | | | | |
| MI | 4 (5.5) | 0 (0.0) | 3 (3.7) | 0.19 | 0.60 | 0.29 |
| Stroke | 6 (7.3) | 1 (3.2) | 1 (1.2) | 0.42 | 0.05 | 0.45 |
| Diabetes | 9 (11.0) | 1 (3.2) | 2 (2.4) | 0.20 | 0.03 | 0.79 |
| Current use of medication | | | | | | |
| Antihypertensives | 31 (37.8) | 3 (9.7) | 18 (21.2) | 0.004 | 0.02 | 0.16 |
| Statins | 16 (19.5) | 2 (6.5) | 13 (15.3) | 0.09 | 0.47 | 0.21 |
| Prednisolone | 27 (32.9) | 4 (12.9) | | 0.03 | | |
| Methotrexate | 28 (34.1) | 9 (29.0) | | 0.61 | | |
| TNF α inhibitors | 24 (30.0) | 5 (16.1) | | 0.14 | | |

Crude mean (SD) values presented and numbers (percentages) for counts.

BMI, body mass index; MI, myocardial infarction; RA, rheumatoid arthritis; smoking, smoking ever vs never; stroke, cerebrovascular accident due to ischaemia or haemorrhage; TNF α , tumour necrosis factor α .

Table 2 Cardiovascular risk markers compared across groups

| Variable | Active RA (n=82) | RA in remission (n=31) | Population controls (n=86) | p Value (active vs remission) | p Value (active vs control) | p Value (remission vs control) |
|------------------------------------|------------------|------------------------|----------------------------|-------------------------------|-----------------------------|--------------------------------|
| Soluble biomarkers | | | | | | |
| CRP (mg/l) | 8.65 (1.49) | 5.35 (2.30) | 3.13 (1.39) | 0.001 | <0.000 | 0.09 |
| Total cholesterol (mmol/l) | 5.22 (0.13) | 5.77 (.20) | 5.84 (0.12) | 0.02 | <0.000 | 0.77 |
| HDL cholesterol (mmol/l) | 1.60 (0.06) | 1.61 (0.09) | 1.72 (0.06) | 0.90 | 0.14 | 0.33 |
| Atherogenic index | 3.58 (0.13) | 3.81 (0.20) | 3.67 (0.12) | 0.23 | 0.17 | 0.24 |
| lnNT-proBNP (pmol/l) | 2.09 (0.11) | 1.99 (0.17) | 2.09 (0.11) | 0.04 | 0.04 | 0.61 |
| Peripheral biomarkers | | | | | | |
| RHI | 2.02 (0.07) | 2.60 (0.11) | 2.13 (0.07) | <0.000 | 0.29 | <0.000 |
| Brachial systolic pressure (mm Hg) | 134.47 (2.01) | 126.47 (3.07) | 127.34 (1.85) | 0.03 | 0.01 | 0.81 |
| Central biomarkers | | | | | | |
| PWV (m/s) | 8.09 (0.16) | 7.31 (0.23) | 7.84 (0.14) | 0.003 | 0.21 | 0.05 |
| Alx | 22.57 (1.05) | 18.35 (1.53) | 18.20 (0.95) | 0.02 | 0.002 | 0.93 |
| Central systolic pressure (mm Hg) | 124.64 (1.94) | 116.20 (2.85) | 117.33 (1.77) | 0.01 | 0.005 | 0.74 |
| IMT (mm) | 0.76 (0.02) | 0.73 (0.03) | 0.74 (0.02) | 0.38 | 0.45 | 0.75 |
| Composite risk score | | | | | | |
| Framingham | 10.93 (0.64) | 10.11 (0.90) | 10.15 (0.53) | 0.44 | 0.34 | 0.97 |
| SCORE | 3.38 (0.27) | 2.46 (0.38) | 2.88 (0.23) | 0.04 | 0.15 | 0.34 |

Estimated marginal mean (SE) presented with adjustments for age and sex.

Alx, augmentation index; BMI, body mass index; CRP, high sensitivity C reactive protein; Framingham, Framingham risk score; HDL cholesterol, high density lipoprotein; IMT, intima-media thickness; lnNT-proBNP, natural logarithm of N-terminal pro-brain natriuretic peptide; PWV, pulse wave velocity; RHI, reactive hyperaemia index; SCORE, European Society of Cardiology SCORE risk calculator.

to 74.80)) removed PWV from the model and gave an improvement in the Nagelkerke value of 0.07, a slight decrease in the sensitivity of 1.3%, without altering the accuracy of the model.

Fifty-five patients were anti-CCP positive, the median vdHSS was 10 (range 0–152) and 13 patients (11.5%) had current or a history of extra-articular manifestations. The distribution of CVD risk markers was similar overall across these categories (table 3).

DISCUSSION

This study shows that patients in RA disease remission score significantly better than patients with active RA across a range of biomarkers predicting CVD. Importantly, the level of CVD risk in this group of RA patients in remission, as judged by these biomarkers, is comparable to that of community controls whereas patients with active RA had worse scores than controls (table 2). Furthermore, we found that a multivariate logistic regression model which included measures of endothelial dysfunction, arterial stiffness and AIx gave the best differentiation between patients with active RA and those with RA in remission. To our knowledge, this is the first study to examine CVD risk in patients in remission according to the CDAI criteria, and the first to compare levels of RHI between patients with RA and community controls.

In this study, systolic pressure in patients with active RA was an average of 8 mm Hg higher than patients in remission. Previous studies on brachial systolic pressure in patients with RA have given conflicting results.^{20–24} A recent interesting post hoc analysis of data from the BeSt trial showed that systolic pressure in patients with RA was correlated to disease activity and reduced by infliximab.²⁵ Our results further showed that patients with active RA had lower total cholesterol than patients with RA in remission and community controls, a finding that has obvious implications for the validity of the calculation of the Framingham score and European Society of Cardiology SCORE in patients with active RA. Indeed, there were no statistically

significant differences in the Framingham score between patient groups and controls, although SCORE was higher in patients with active RA (table 2). The use of SCORE may be advantageous for our patients for two reasons: it can be calculated using the atherogenic index (dividing the total cholesterol by the high density lipoprotein) and it is based on European populations. Heterogeneous findings concerning the lipid profiles of patients with RA have been reported. Adverse lipid profiles have been found in cohorts across a range of disease durations and activity states.^{26–27} However, RA and chronic inflammation have also been shown to correlate with reduced total cholesterol levels (high density, low density and very low density lipoproteins), possibly due to increased clearance of low density lipoprotein cholesterol by the reticuloendothelial system or decreased lipoprotein lipase activity.^{22–28–31}

The RHI is a measure of endothelial function which correlates with the gold standard of coronary endothelial function measurements and was recently shown to predict cardiovascular events in a prospective study.^{14–32} Flow-mediated dilation, the classic method of non-invasive endothelial function assessment, has been found to be decreased in patients with RA.^{33–34} Endothelial dysfunction may impact on arterial stiffness through nitric oxide, which is important in arterial stiffness regulation.³⁵ Inflammatory cytokines may also act through upregulation of angiotensin type 1 receptors to cause vasoconstriction and hypertension.^{24–36} Furthermore, an injury to the endothelium can proceed to intimal thickening with a decrease in vascular wall contractile elements as smooth muscle cells migrate to the intima, multiply and lay down extracellular matrix.³⁷ The resultant arterial stiffening is characterised by increased vascular collagen formation, calcification and breakdown of elastin.¹⁵

Carotid-femoral PWV pulse wave velocity, AIx and central systolic pressures are measures of arterial stiffening and wave reflection that have been used to predict cardiovascular morbidity and mortality in numerous studies.^{15–38–41} The AIx and central pressure estimations quantify the pressure amplification

Table 3 Comparison of cardiovascular risk markers across three patient categorisations†

| Cohorts | Anti-CCP positive (n=55) | Anti-CCP negative (n=51) | p Value | Joint erosions >median (n=54) | Joint erosions ≤median (n=53) | p Value | Extra-articular manifestations (n=13) | No extra-articular manifestations (n=100) | p Value |
|------------------------------------|--------------------------|--------------------------|---------|-------------------------------|-------------------------------|---------|---------------------------------------|---|---------|
| Soluble biomarkers | | | | | | | | | |
| CRP (mg/l) | 9.05 (1.71) | 5.26 (1.82) | 0.06 | 9.73 (2.12) | 8.67 (2.05) | 0.34 | 16.82 (3.45) | 4.88 (0.98) | <0.000* |
| Total cholesterol (mmol/l) | 5.20 (0.16) | 5.47 (0.17) | 0.22 | 5.12 (0.17) | 5.60 (0.17) | 0.03* | 5.18 (0.31) | 5.62 (0.09) | 0.16 |
| HDL cholesterol (mmol/l) | 1.66 (0.08) | 1.60 (0.09) | 0.55 | 1.59 (0.08) | 1.67 (0.09) | 0.46 | 1.69 (0.14) | 1.65 (0.04) | 0.79 |
| Atherogenic index | 3.45 (0.15) | 3.72 (0.16) | 0.17 | 3.35 (0.17) | 3.85 (0.16) | 0.02* | 3.34 (0.31) | 3.68 (0.09) | 0.27 |
| lnNT-proBNP (pmol/l) | 2.43 (0.12) | 2.27 (0.13) | 0.30 | 2.49 (1.26) | 2.15 (1.23) | 0.03* | 2.53 (0.26) | 2.19 (0.08) | 0.19 |
| Peripheral biomarkers | | | | | | | | | |
| RHI | 2.13 (0.10) | 2.28 (0.11) | 0.30 | 2.06 (0.11) | 2.30 (0.11) | 0.08 | 2.05 (0.18) | 2.17 (0) | 0.44 |
| Brachial systolic pressure (mm Hg) | 133 (2.4) | 133 (2.4) | | 132 (2.6) | 135 (2.5) | 0.28 | 136 (4.8) | 123 (1.3) | 0.20 |
| Central biomarkers | | | | | | | | | |
| PWV | 8.02 (0.19) | 8.09 (0.21) | 0.77 | 7.88 (0.22) | 8.24 (0.20) | 0.18 | 8.38 (0.35) | 7.82 (0.10) | 0.12 |
| AIx | 22.84 (1.33) | 22.55 (1.43) | 0.87 | 23.86 (1.39) | 22.98 (1.31) | 0.61 | 23.17 (2.44) | 19.74 (0.71) | 0.17 |
| Central systolic pressure (mm Hg) | 124 (2.5) | 124 (2.7) | 0.99 | 122 (2.7) | 125 (2.5) | 0.36 | 125 (4.7) | 120 (1.4) | 0.33 |
| IMT (mm) | 0.74 (0.02) | 0.75 (0.02) | 0.74 | 0.75 (0.02) | 0.76 (0.02) | 0.55 | 0.73 (0.04) | 0.74 (0.01) | 0.66 |
| Composite score | | | | | | | | | |
| Framingham | 9.99 (1.06) | 9.54 (1.17) | 0.78 | 10.96 (0.96) | 12.29 (0.92) | 0.26 | 8.58 (1.69) | 10.54 (0.40) | 0.25 |
| SCORE | 3.42 (0.29) | 9.3.23 (0.32) | 0.64 | 3.77 (0.32) | 3.21 (0.30) | 0.15 | 2.60 (0.69) | 2.98 (0.17) | 0.59 |

Estimated marginal mean (SE) presented with adjustments for age and sex.

*p<0.05.

†The following three categorisations were compared: anti-CCP positive vs negative; joint erosions >median vs ≤median joint erosions; and presence of extra-articular manifestations vs no extra-articular manifestations.

AIx, augmentation index; CRP, high sensitivity C reactive protein; Framingham, Framingham risk score; HDL cholesterol, high density lipoprotein; IMT, intima-media thickness; lnNT-proBNP, natural logarithm of N-terminal pro-brain natriuretic peptide; PWV, pulse wave velocity; RHI, reactive hyperemia index; SCORE, European Society of Cardiology SCORE risk calculator.

caused by wave reflection and have been shown to be superior to brachial pressure in predicting CVD.³⁸ AIx^{42–44} and PWV^{45–46} are both reported to be increased in patients with high levels of inflammation. AIx has also been shown to be predicted by cumulative scores of disease activity,^{43–44} whereas PWV—rather than AIx—is reduced by treatment with TNF α inhibitors.^{45–47–48} These heterogeneous findings may partly be explained by the peripheral vasodilation that accompanies acute inflammation, reducing pulse wave reflection and thereby the AIx and central systolic pressures.⁴⁹

NT-proBNP is an independent predictor of cardiovascular morbidity and also general mortality in several populations including patients with RA.^{50–51} NT-proBNP levels reflect the stretching of the atria and ventricles and are associated with measures of arterial stiffness,⁵² but this biomarker is also upregulated by proinflammatory cytokines.⁵³ In patients with RA, NT-proBNP levels correlate with CRP and are reduced by TNF α inhibitors.^{54–55}

The IMT is a direct measure of the atherosclerotic disease process,⁵⁶ and an increased IMT predicts a greater risk of cardiovascular events.⁵⁷ A recent meta-analysis of IMT in patients with RA included 25 studies of which 19 reported increased IMT in patients with RA, and it is thus surprising that our study did not find that patients with active RA had significantly higher IMT compared with the other groups.⁵⁸

We chose to categorise RA disease activity according to the CDAI criteria for active RA versus remission as it is a more stringent categorisation than the DAS criteria.¹⁰ Since CRP is a marker of both RA disease activity and CVD risk, we found it advantageous to categorise patients by a composite score that does not include CRP as a component. Verification of RA disease remission by long-term radiographic outcome data would, however, have added validity to our findings. A weakness of our study is the low participation rate among the invited controls, which may challenge our findings through a selection bias. The controls participating in our study had a poorer CVD risk profile with regard to RHI and PWV compared with patients in remission, which may suggest that some controls chose to participate as they felt the need for a CVD risk assessment. Such a bias would make a type I statistical error unlikely, but a type II error may have occurred. This is a cross-sectional study and our findings should be confirmed in a longitudinal study, preferably with incident CVD or mortality as the outcome.

This study indicates that classification of RA disease activity state according to the CDAI gives better differentiation between the groups than a comparison of CVD risk according to anti-CCP status, level of joint destruction or extra-articular manifestations (tables 2 and 3). Our results indicate that patients with active RA should receive extra attention from clinicians with regard to CVD prevention.

In conclusion, patients with active RA—but not those in remission—have significantly increased levels of biomarkers for CVD. These results support the association of inflammatory activity and markers of CVD risk in patients with RA and indirectly support the finding that remission in RA may also confer diminished cardiovascular morbidity.

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Competing interests None.

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NT-proBNP predicts mortality in patients with rheumatoid arthritis: results from 10-year follow-up of the EURIDISS study

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ABSTRACT

Objectives Patients with rheumatoid arthritis (RA) have a higher mortality than the general population, and this increased mortality is related to demographic and disease variables. N-terminal pro-brain natriuretic peptide (NT-proBNP) is a predictor of mortality both in general and patient populations, but has not been shown to predict mortality in patients with RA. This study examines whether NT-proBNP can further improve the prediction of mortality in RA.

Methods 182 patients with RA of 5–9 years disease duration were comprehensively examined in 1997. Serum samples were frozen and later batch analysed for NT-proBNP levels and other biomarkers. Adjusted univariate and logistic regression analyses were performed with death within the 10-year follow-up period as the dependent variable. Significant predictors were also examined as dichotomised variables.

Results Mortality was predicted in univariate analyses by the following variables: age, sex, homozygosity for HLA-DRB1 shared epitope alleles, Health Assessment Questionnaire, 28-joint Disease Activity Score (DAS28) and NT-proBNP. A multivariate model with age, sex, DAS28 and NT-proBNP as independent variables showed the greatest discrimination.

Conclusion NT-proBNP provided incremental information in the prediction of mortality in this cohort of patients with RA.

INTRODUCTION

Mortality in patients with rheumatoid arthritis (RA) is increased compared with the general population by at least 50%.^{1–3} The excess mortality is largely due to cardiovascular disease (CVD), but malignancies and infections are also increased.¹ The absolute risk of death from CVD is highest for elderly men with RA whereas the relative risk is highest for young women.⁴ Established predictors for all-cause mortality in patients with RA are age, male sex, physical function, comorbidities and rheumatoid factor (RF).^{1–5} A homozygote shared epitope (SE) genotype has been found to be associated with premature death, and specifically death from ischaemic heart disease.^{6–8} Inflammatory markers such as C reactive protein (CRP) and erythrocyte sedimentation rate (ESR) have also been shown to be important risk indicators in RA, pertaining to general mortality, CVD mortality and cardiovascular events.^{9–10}

The level of N-terminal pro-brain natriuretic peptide (NT-proBNP) predicts all-cause and cardiac mortality in the general population, as well as in cohorts of patients with heart failure and stable

coronary heart disease.^{11–14} NT-proBNP has been found to be increased in patients with RA and is cross-sectionally associated with the level of inflammation.^{15–16} As far as we know, NT-proBNP has not been investigated as a predictor of mortality in patients with RA.

Our hypothesis was that NT-proBNP is an independent predictor of 10-year mortality in patients with RA. We have thus examined the incremental value of NT-proBNP in the prediction of mortality in RA across a panel of established and potentially novel risk factors.

MATERIAL AND METHODS

Patient cohort

One hundred and eighty-two patients with RA participated in the 5-year follow-up of the EURIDISS study in 1997. Data from this comprehensive visit are used as the baseline for the analyses in this paper.¹⁷ The patients were diagnosed according to the 1987 American College of Rheumatology criteria.¹⁸ The mean age at baseline was 55.7 years (range 27.3–74.7), mean disease duration was 7.1 years (range 4.76–9.4) and 134 (74%) were women. Eighteen patients (17%) reported previous CVD. At the 10-year follow-up in 2007, 31 (17%) of the patients had died.

Baseline variables 1997

A questionnaire recording data on demographics, comorbidities and health status was completed by patients in collaboration with the study nurse. Health status was measured by the Stanford Health Assessment Questionnaire (HAQ), a multidimensional questionnaire that captures patient functioning in several areas of daily life,¹⁹ and by the generic Nottingham Health Profile (NHP).²⁰ A checklist modified from Arthritis Impact Measurement Scales was used to assess the presence of 16 possible comorbidities including patient self-reported presence of hypertension, angina, myocardial infarction or other cardiac diseases and diabetes, without any diagnostic specifications.²¹ The trained study nurse also performed a clinical examination for the presence of nodules and joint swelling and the composite Disease Activity Score was calculated (DAS28 consisting of tender joint score, swollen joint score, ESR and patient visual analogue scale for global assessment).²² Biomarkers were examined from blood (DNA) and serum samples that had been frozen at –70°C: anti-cyclic citrullinated antibody (anti-CCP) by ELISA (Inova Diagnostics, San Diego, California, USA), high sensitivity CRP

by phyCardioPhase, nefelometry (Dade Behring, Deerfield, Illinois, USA) and RF IgM using the in-house ELISA method.

NT-proBNP was measured using a Modular E170 device (Roche Diagnostics, Mannheim, Germany). The coefficient of variation on batch analyses was 1.4%. DRB1 sequencing was performed using BigDye Terminator V3.1 chemistry (Applied Biosystems, Foster City, California, USA) on an ABI 3730 DNA Analyzer (Applied Biosystems) followed by allele assignment (AssignSBT V3.2.7; Conexio Genomics, Applecross, Australia). SE was defined as the following alleles: 0101, 0102, 0401, 0404, 0405, 0408, 1001 and 1402.

Haemoglobin and ESR were analysed at the time of examination.

Outcome measures

The patients were registered as dead at the 2007 follow-up study on the basis of information from the Norwegian National Population Register.

Statistical analyses

Baseline demographic disease activity, comorbidities, health status variables and levels of soluble biomarkers were compared between patients who had died at the 10-year follow-up and the survivors using the χ^2 test, Student *t* test or Mann-Whitney *U* test as appropriate. Two-tailed *p* values are reported.

The associations between baseline variables and 10-year mortality were examined in a logistic regression procedure that was adjusted for age and sex. Variables that were significantly skewed were transformed into the natural logarithm (CRP, ESR, NT-proBNP) or dichotomised at the median (NHP and HAQ). Anti-CCP and IgMRF levels were classified as elevated if ≥ 25 U/ml. Separate models were constructed for associated variables. Finally, a multivariate stepwise backward regression model was constructed that initially included all variables that were significant at the $p \leq 0.15$ level. The validity of the final model was confirmed by performing sensitivity analyses with NT-proBNP dichotomised according to the age and sex-specified 95th percentile in healthy blood donors,²⁵ and by forcing baseline CRP and history of CVD into the model. Previously excluded variables of baseline disease activity were also re-entered into the final model to check for possible confounding. To verify that the models were not overfitted, variables that were significant predictors of mortality in the separate models were dichotomised at the median and, for each predictor, a sum variable was constructed that also included age and sex (eg, age > median = 1 + female sex = 1 + HAQ \leq median = 0). NT-proBNP was then added to the logistic models that included one such sum variable and improved prediction was assessed by examining the CI of the receiver operating characteristics (ROC) curves. The relationship between NT-proBNP and mortality was additionally explored using fractional polynomial analysis in STATA. In addition, we checked for model lack of fit by applying the Hosmer-Lemeshow goodness-of-fit test to the final model and, finally, we plotted Cook's distances and repeated the final step of the analyses after the removal of outliers. When not otherwise mentioned, the analyses were performed using SPSS Version 14.

RESULTS

Of the 182 patients, 31 had died during the 10-year follow-up period. Details of missing data are given in table 1. Complete baseline data for variables included in the final model were

available for 117 patients, 21 of whom had died during the 10-year follow-up period. These patients were similar to the main cohort with regard to age and level of IgM RF, anti-CCP, HAQ and DAS28, but fewer women had participated in the complete data collection (complete data were available for 76.6% of men vs 60.0% of women, $p=0.04$).

Bivariate analyses

As expected, the patients who had died were older, more often male, had a higher frequency of CVD-related comorbidities and poorer functional status (HAQ and NHP) at baseline. They also had higher baseline levels of DAS28, and NT-proBNP, CRP and ESR levels were borderline significantly increased in patients who died ($p=0.08$ and $p=0.06$, respectively; table 1).

Logistic regression

Significant and borderline predictors of 10-year mortality are shown in table 2. In the demographic model, homozygous SE adjusted for age and sex correctly predicted 8 of 29 deaths. Level of education was not a significant predictor of mortality (OR 0.90, 95% CI 0.32 to 2.49). Among the variables of disease activity, only DAS28 was a significant predictor with a prediction of 10 of 30 deaths. The rejected variables were nodules (OR 1.20, 95% CI 0.39 to 3.65), anti-CCP (OR 1.60, 95% CI 0.54 to 4.77) and IgM RF (OR 0.85, 95% CI 0.30 to 2.37).

None of the comorbidities were significant predictors at $p \leq 0.05$, although a history of myocardial infarction approached this level. Neither hypertension (OR 1.32, 95% CI 0.45 to 3.85) nor diabetes (OR 1.11, 95% CI 0.26 to 4.77) contributed to the model. The dichotomised variables reflecting health status (NHP and HAQ) were both significant in the adjusted analysis, but NHP was surpassed by HAQ in a multivariate 'health status model' and lost significance. A high NHP score adjusted for age

Table 1 Comparison of baseline variables between patients who were dead (n=31) and those who were alive (n=151) after 10 years of follow-up

| Baseline variables (1997) | Deceased by 2007 | Alive in 2007 | p Value |
|--------------------------------------|------------------|---------------|---------|
| Demographic variables (n=182) | | | |
| Age | 62.0 (0.9) | 48.4 (1.0) | <0.001 |
| Female sex | 19 (59.4) | 120 (76.9) | 0.04 |
| Education high vs low | 8 (25.0) | 50 (32.1) | 0.43 |
| RA disease variables | | | |
| DAS28 (n=172) | 5.0 (0.3) | 3.87 (0.12) | <0.001 |
| Presence of nodules (n=181) | 6 (19.4) | 22 (14.9) | 0.53 |
| Comorbidities (n=178) | | | |
| Hypertension | 8 (25.8) | 13 (8.7) | 0.01 |
| MI | 9 (30.0) | 9 (6.1) | <0.001 |
| Diabetes | 3 (9.7) | 11 (7.4) | 0.67 |
| Health status | | | |
| HAQ (n=179) | 1.3 (0.1) | 0.8 (0.1) | <0.001 |
| NHP total (n=176) | 2.0 (0.1) | 1.5 (0.1) | <0.001 |
| Biomarkers | | | |
| SE homozygote (n=168) | 12 (40.0) | 35 (24.3) | 0.08 |
| CRP (n=127) | 13.5 (16.4) | 8.6 (10.4) | 0.08 |
| ESR (n=177) | 25.8 (3.7) | 18.2 (1.2) | 0.06 |
| NT-proBNP (n=124) | 61.0 (79.8) | 11.2 (8.7) | <0.001 |
| Haemoglobin (n=177) | 13.7 (0.3) | 14.5 (0.1) | 0.54 |
| Anti-CCP level (n=127) | 140 (23) | 118 (11) | 0.42 |
| IgMRF level (n=127) | 95 (23) | 71 (9) | 0.41 |

Mean (SE) for continuous variables, numbers (%) for categorical variables. anti-CCP, anti-cyclic citrullinated peptide antibody; CRP, high sensitivity C reactive protein; DAS28, Disease Activity Score for 28 joints; ESR, erythrocyte sedimentation rate; HAQ, Stanford Health Assessment Questionnaire; IgMRF, rheumatoid factor IgMRF; MI, myocardial infarction; NHP, Nottingham Health Profile; NT-proBNP, N-terminal pro-brain natriuretic peptide; SE, shared epitope.

Table 2 Logistic regression models for the prediction of death during 10-year follow-up (OR (CI) and p value)

| | Demographic model (n=168) | Disease activity model (n=172) | Comorbidities model (n=178) | Health status model (n=178) | Biomarker model (n=124) | Final model (n=117) |
|---------------|--------------------------------|--------------------------------|--------------------------------|---------------------------------|-----------------------------------|-----------------------------------|
| Age | 1.16 (1.08 to 1.24) p<0.001 | 1.15 (1.07 to 1.23) p<0.001 | 1.14 (1.07 to 1.23) p<0.001 | 1.16 (1.08 to 1.25) p<0.001 | 1.01 (1.01 to 1.21) p=0.02 | 1.16 (1.08 to 1.24) p<0.001 |
| Female sex | 0.56 (0.20 to 1.51) p=0.25 | 0.43 (0.16 to 1.14) p=0.09 | 0.58 (0.23 to 1.51) p=0.27 | 0.47 (0.18 to 1.27) p=0.14 | 0.46 (0.14 to 1.49) p=0.20 | 0.46 (0.13 to 1.67) p=0.27 |
| SE homozygote | 3.01 (1.09 to 8.36) p=0.03 | | | | | |
| DAS28 | | 1.57 (1.11 to 2.22) p=0.01 | | | | 1.67 (1.06 to 2.65) p=0.03 |
| Previous MI | | | 2.59 (0.81 to 8.27) p=0.11 | | | |
| HAQ | | | | 4.88 (1.78 to 13.43) p=0.002 | | |
| LnNT-ProBNP | | | | | 26.85 (3.56 to 202.40) p=0.001 | 24.23 (2.85 to 206.02) p=0.004 |
| Accuracy | 85.1 | 84.9 | 87.1 | 89.3 | 88.1 | 89.7 |
| Sensitivity | 27.6 | 33.3 | 36.7 | 53.3 | 52.2 | 66.7 |
| Specificity | 97.1 | 95.8 | 97.3 | 96.6 | 96.1 | 94.8 |

DAS28, Disease Activity Score for 28 joints; HAQ, Stanford Health Assessment Questionnaire; MI, myocardial infarction; NT-proBNP, N-terminal pro-brain natriuretic peptide; SE, shared epitope.

and sex predicted 7 of 27 deaths (OR 3.22, 95% CI 1.18 to 8.80) whereas a higher HAQ score predicted 16 of 30.

NT-proBNP was found to be an independent predictor in the 'biomarker model' predicting 12 of 23 deaths. LnCRP was also a near-significant predictor of death in the age and sex-adjusted model (OR 1.48, 95% CI 0.95 to 2.31) but did not perform when NT-proBNP was included in the model. LnESR (OR 1.42, 95% CI 0.80 to 2.51) did not carry significance in the model.

The final model included DAS28 and NT-proBNP and predicted 14 of 21 deaths. The positive predictive value of the model was 74% and the negative predictive value was 93%. The ROC curves comparing the final model with the health status model showed that the combination of DAS28 and NT-proBNP had the greatest area under the curve (AUC), although the CIs were overlapping and the difference was thus not significant (figure 1).

The sensitivity analyses supported the main results. NT-proBNP categorised according to the age and sex-specific 95th percentile provided by the manufacturer in package inserts (based on samples from 2264 blood donors and patients aged between 18 and 65 and free from CVD)²³ was a slightly poorer predictor of death than the continuous variable (sensitivity 62%, specificity 95%, accuracy 89%). The final model was not significantly altered by forcibly re-entering previously rejected variables (including self-reported CVD and hypertension), and no other variables were found to be significant predictors of the outcome nor to be confounders of the included variables. Consecutive models that each included one sum variable showed that NT-proBNP remained a significant predictor of mortality and contributed significantly to each model (data not shown). According to the fractional polynomial analyses, the relationship between NT-proBNP and death was best described by a linear equation. The model exhibited no evidence of lack of fit (Hosmer–Lemeshow, p=0.46) and was not substantially changed after the removal of three outliers.

DISCUSSION

Our results show that NT-proBNP is an independent predictor of 10-year mortality in this cohort of patients with longstanding RA. Established risk factors for RA mortality were also significant predictors in our cohort and the HAQ score, adjusted for age and sex, had the highest single variable discriminatory power.

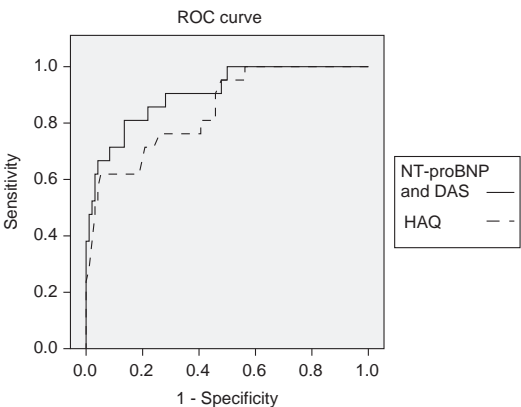


Figure 1 Receiver operating characteristics (ROC) curves comparing the performance of two models predicting mortality after 10 years. Models adjusted for age and sex. Area under the curve for N-terminal pro-brain natriuretic peptide (NT-proBNP) and Disease Activity Score (DAS) model 0.91, for Stanford Health Assessment Questionnaire (HAQ) model 0.85.

Biomarkers are quantifiable biological parameters that serve as important indices of healthy or pathological processes.²⁴ Biomarkers reflecting RA disease activity can be grouped into two clusters, the variables in each cluster being mutually closely correlated. One cluster includes variables that describe patient-centred outcomes; these may be derived from patient self-reported questionnaires of which the HAQ score is an example. The other cluster includes measures of RA disease-centred outcomes such as ESR, CRP, RF and radiographic joint erosions. In general, patient-centred outcomes are reported to be better predictors of mortality than disease-centred variables¹ although, to our knowledge, they have not previously been compared with SE or NT-proBNP. NT-proBNP as a single variable (adjusted for age and sex) had approximately the same predictive ability as the HAQ score. The final model that included DAS28 was superior to other models, although the CIs of the ROC curves were not significantly different.

NT-proBNP is a validated marker of heart failure that has been shown to complement traditional risk factors as an independent predictor of all-cause mortality in a community cohort.²⁵ This biomarker is the biologically inactive N-terminal fragment of the active hormone BNP and is part of a family of natriuretic peptides that cause vasodilation, natriuresis and smooth muscle modulation in response to myocardial stretch.²⁶ NT-proBNP and BNP release may also be upregulated by proinflammatory cytokines.²⁷ It is thus possible that measures of inflammation or RA disease activity could attenuate the link between NT-proBNP and mortality. In the failing heart, NT-proBNP levels can accordingly be reduced by medication that improves cardiac function²⁸ and, interestingly, a recently published paper found that NT-proBNP is also reduced in patients with RA by treatment with a tumour necrosis factor α inhibitor.²⁹ In our final model, NT-proBNP was maintained as a significant predictor of mortality when self-reported CVD, self-reported hypertension and current inflammation were forcibly imputed into the model.

The observed yearly all-cause mortality in this cohort was 1.7%. A 4% yearly mortality has been observed in another Norwegian study on data collected from 1977 to 1992, two decades prior to our inclusion date.³ Our observed yearly mortality was also lower than the 2.4–2.5% observed in the Minnesota cohort, although our data are within the CIs of the Minnesota findings.³⁰ The average age in these cohorts seems comparable, and we believe that the lower mortality observed in our study might be explained by the more recent data collection, mirroring the reduced mortality seen in the general population. The EURIDISS study is an inception cohort that specifically included patients with >5 and <10 years of disease duration and, as death in RA has been found to be increased early in the disease course, this may have biased our results towards a lower mortality.³¹

One strength of our study is the prospective longitudinal design with subsequent batch analyses of baseline soluble biomarkers. The results of this study confirm several previous findings concerning predictors of mortality in RA, providing validity to our findings. The main weakness of this study is the sample size which may lead to a type II statistical error regarding less powerful predictors. Hypertension and presence of diabetes were not significant predictors of mortality in our models, and this may well be due to the low number of patients with these conditions. We chose to use the 1997 examination as the baseline as information on CVD had been collected at this time point. However, we do not have details of ventricular function, renal function, lipid levels or smoking habits from this visit, all of which are possible predictors and confounders. Ideally we should have entered NT-proBNP into multivariate models that already included composite and extensively validated scores, such as the Framingham score, in order to discern whether NT-proBNP can further improve prediction of mortality in addition to these widely used predictors. Lack of knowledge about the cause of death prohibited subgroup analyses but, because of the limited number of patients, the cohort would not have been eligible for robust subgroup analyses according to cause of death.

In summary, NT-proBNP compared favourably with the established predictors of mortality in this cohort of patients with RA. The resultant model gave an improved sensitivity, specificity and the highest AUC of the ROC analysis. Additional studies are encouraged to replicate and further explore the value of NT-proBNP as a prognostic biomarker in RA.

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Competing interests None.

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